

## Research Article

# A Fast and Validated HPLC Method for Simultaneous Determination of Dopamine, Dobutamine, Phentolamine, Furosemide, and Aminophylline in Infusion Samples and Injection Formulations

Fuchao Chen <sup>1,2</sup>, Baoxia Fang <sup>2</sup>, and Sicen Wang <sup>1,2</sup>

<sup>1</sup>School of Pharmacy, Xi'an Jiaotong University, Xi'an 710061, Shanxi, China

<sup>2</sup>Sinopharm Dongfeng General Hospital, Hubei University of Medicine, Shiyuan, Hubei 442008, China

Correspondence should be addressed to Sicen Wang; wangsc@mail.xjtu.edu.cn

Received 31 July 2020; Revised 30 November 2020; Accepted 11 February 2021; Published 27 February 2021

Academic Editor: Serban C. Moldoveanu

Copyright © 2021 Fuchao Chen et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

A simple, fast, and validated HPLC method was developed for the simultaneous quantization of five cardiovascular agents: dopamine (DPM), dobutamine (DBM), phentolamine (PTM), furosemide (FSM), and aminophylline (APL) either in infusion samples or in an injection dosage form. The proposed method was achieved with a 150 mm × 4.6 mm, 5.0 μm C<sub>18</sub> column, by using a simple linear gradient. Mobile phase A was buffer (50 mM KH<sub>2</sub>PO<sub>4</sub>) and mobile Phase B was acetonitrile at a flow rate of 1.0 mL/min. The column temperature was kept at 30°C, and the injection volume was 20 μL. All analytes were separated simultaneously at a retention time (tr) of 3.93, 5.84, 7.06, 8.76, and 9.67 min for DPM, DBM, PTM, FSM, and APL, respectively, with a total run time of less than 15.0 min. The proposed method was validated according to ICH guidelines with respect to accuracy, precision, linearity, limit of detection, limit of quantitation, and robustness. Linearity was obtained over a concentration range of 12.0–240.0, 12.0–240.0, 20.0–200.0, 6.0–240.0, and 10.0–200.0 μg/mL DPM, DBM, PTM, FSM, and APL, respectively. Interday and intraday accuracy and precision data were recorded in the acceptable limits. The new method has successfully been applied for quantification of all five drugs in their injection dosage form, infusion samples, and for evaluation of the stability of investigated drugs in mixtures for endovenous use. The results of the stability study showed that mixtures of DPM, DBM, PTM, FSM, and APL in 5% glucose or 0.9% sodium chloride injection were stable for 48 hours when stored in polypropylene syringes at 25°C.

## 1. Introduction

Congestive heart failure (HF) is an important health care problem across the world [1]. The prevalence of HF is 1–2% in developed countries and is expected to rise even further in the next decades [2, 3]. Diuretic therapy is an essential part of the management of the majority of patients with HF [4]. Furosemide (FSM, Figure 1(a)), 4-chloro-2-((furan-2-ylmethyl) amino)-5-sulfamoylbenzoic acid, a loop diuretic drug, is frequently administered to increase urinary output [5–10]. However, diuretic resistance is common in patients with acute HF and is associated with an increased risk of morbidity and mortality. The current strategies to overcome diuretic resistance include restriction of sodium intake,

coadministration regimens, continuous diuretic infusions, and mechanical ultrafiltration [11, 12].

Dopamine (DPM, Figure 1(b)), 2-(3, 4-dihydroxyphenyl) ethylamine, is an endogenous substance with dose-dependent effects. Dobutamine (DBM, Figure 1(c)), 4-(2-(3-(4-hydroxyphenyl)-1-methyl-propylamino)-ethyl)-benzene-1,2-diol, a beta-1 agonist catecholamine has cardiac stimulant action without evoking vasoconstriction or tachycardia. Phentolamine (PTM, Figure 1(d)), 3-((4, 5-dihydro-1H-imidazol-2-ylmethyl) (4-methylphenyl) amino) phenol, is a long-acting, adrenergic, nonselective alpha-receptor blocking agent. Aminophylline (APL, Figure 1(e)), bis(1, 3-dimethyl-2,3,6,7-tetrahydro-1H-purine-2,6-dione), ethane-1, 2-diamine, is the ethylenediamine salt of theophylline. It relaxes certain smooth

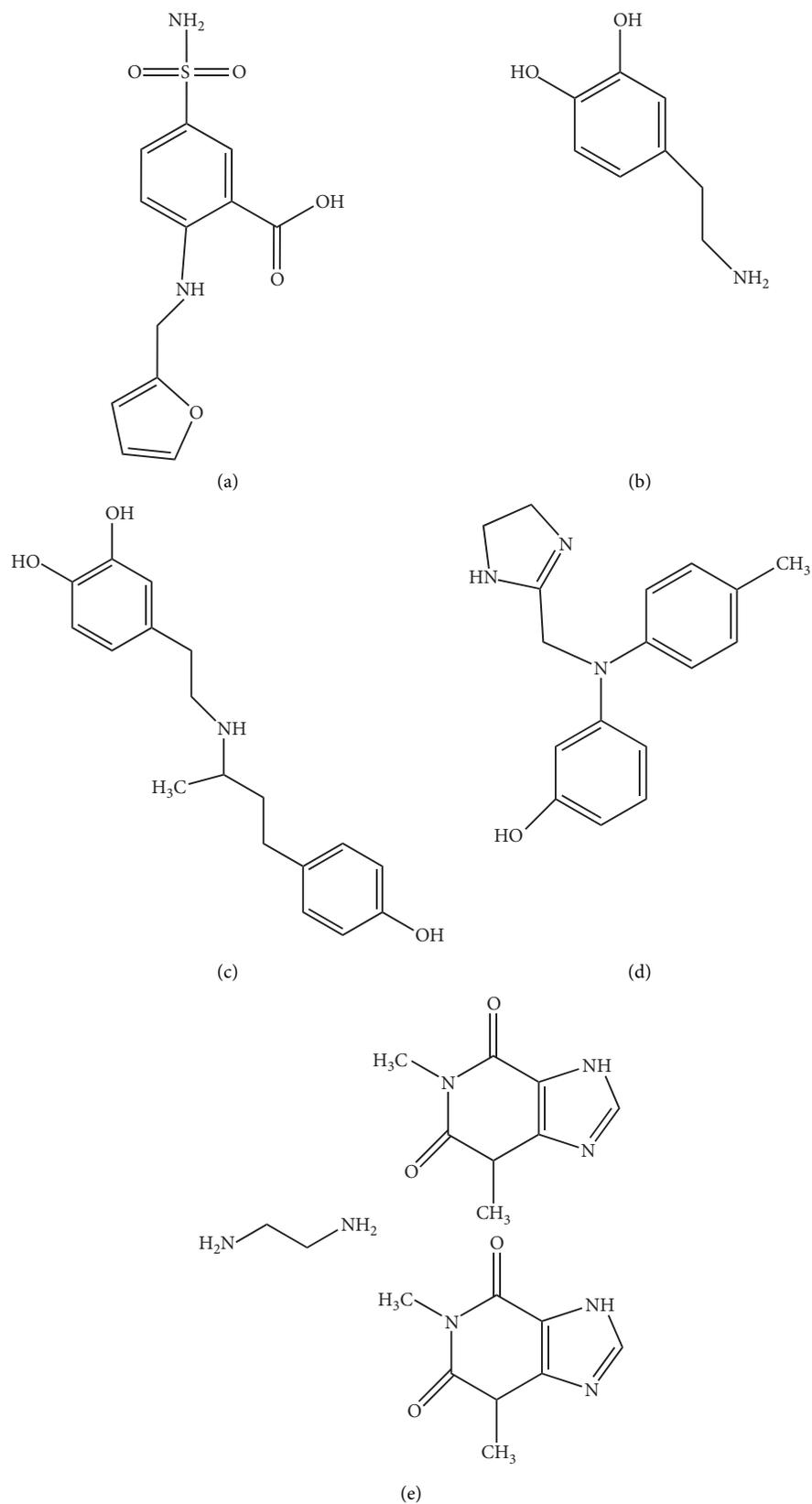


FIGURE 1: Chemical structure of FSM (a), DPM (b), DBM (c), PTM (d), and APL (e).

muscles in the bronchi, produces diuresis, and causes an increase in gastric secretion. Previous guidelines and studies for HF management have suggested that the addition of DPM, DBM, PTM, and APL to diuretic therapy enhances decongestion and preserves renal function during diuretic therapy [13–18]. In addition, coadministration of diuretic therapy has become an accepted clinical practice for HF patients in our institution. However, there are no commercially available such drug mixtures, and they must be prepared in a centralized preparation service department for clinical use. Therefore, specific information on drug stability in various solutions, individually or associated, is necessary to ensure patient's safety. Analytical methods for their quality control are interesting not only as part of hospital-based quality control of the five cardiovascular agents in their injection dosage form but also for drug stability study and intravenous admixture preparation error monitoring.

By reviewing the literature, various analytical methods have been developed for individual quantification of DPM, DBM, PTM, FSM, or APL, or in combination with other drugs by HPLC [19–29]. Our study endeavors to develop and validate a reversed-phase HPLC method for simultaneous quantification of DPM, DBM, PTM, FSM, and APL in infusion samples and in the injection dosage form. To the extent of our knowledge, no analytical method based on reversed-phase HPLC has been reported so far for the simultaneous estimation of the mixture containing DPM, DBM, PTM, FSM, and APL. Therefore, the current study was aimed to develop a rapid, simple, and reproducible reversed-phase HPLC method for simultaneous quantification of the above five cardiovascular drugs in infusion samples and in injection dosage form and to determine the stability of the five cardiovascular drugs, diluted with 0.9% sodium chloride or 5% glucose injection and packaged in polypropylene syringes for 48 hours (2 days) at 25°C.

## 2. Materials and Methods

**2.1. Chemicals and Reagents.** The reference standards of DPM, DBM, PTM, FSM, and APL were procured from the National Institute for Control of Pharmaceutical and Biological Products (Beijing, PR China) and stored at 4°C. Potassium dihydrogen phosphate  $\text{KH}_2\text{PO}_4$  (AR grade) was supplied by Shenzhen Yihao Technology Development Co., Ltd. (Guangdong, China). Water (HPLC -grade) was prepared in our laboratory using a flow water purification system (Millipore Corp., USA). Acetonitrile (HPLC-grade) was purchased from Fisher Scientific International (St Louis, MO, USA). The marketed formulations of drug used in this study were DPM hydrochloride injection 20 mg/2 mL (Harvest Pharmaceutical Co., Ltd., Shanghai, PR China), DBM hydrochloride injection 20 mg/2 mL (Shanghai No. 1 Biochemical and Pharmaceutical Co. Ltd., Shanghai, PR China), PTM mesylate injection 20 mg/2 mL (Beijing Novartis Pharmaceutical Co. Ltd., Beijing, PR China), FSM injection 20 mg/2 mL (Harvest Pharmaceutical Co., Ltd., Shanghai, PR China), and APL injection 20 mg/2 mL (Henan Lingrui Pharmaceutical Co., Ltd., Xinyang, PR China). The commercial solution of 0.9% sodium chloride injection was

purchased from Kelun Pharmaceutical Co., Ltd. (Sichuang, China).

**2.2. Instrumentation.** The HPLC system used for the optimized method was a UltiMate3000 LC (Thermo Fisher) consisting of a column oven, a quaternary pump, an automatic injector, and a diode array detector. The system was controlled by Chromeleon 7.2 software (all from Thermo Fisher).

**2.3. Chromatographic Conditions.** A SinoChrom ODS-BP column (4.6 mm  $\times$  150 mm i.d. and 5.0  $\mu\text{m}$  particle size) was used as the stationary phase. Mobile phase A was 50 mM  $\text{KH}_2\text{PO}_4$  buffer, and mobile phase B was acetonitrile with simple gradient program (0–5 min: MP-A: 95–65; 5–10 min: MP-A: 65–65; 10–11 min: MP-A: 65–95; 11–15 min: MP-A: 95–65) was delivered at a flow rate of 1.0 mL/min. The mobile phase was prepared daily and filtered with a 0.45  $\mu\text{m}$  membrane filter (Millipore Corp., USA). The column temperature was set at 30°C. And, samples were analyzed at a wavelength of 280 nm and injected at 20  $\mu\text{L}$  injection volume.

**2.4. Preparation of Stock, Working, and Standard Curve Solutions.** The standard stock solutions (0.6 mg/mL) of DPM, DBM, and FSM and (1.0 mg/mL) of PTM and APL were prepared by dissolving an accurately weighed 6 or 10 mg of each reference standards separately in 10 mL of deionised water in a 10 mL volumetric flask. The solutions were kept at 20°C until use.

For the linearity studies, a 6-point calibration curve was prepared by diluting the working standard solution with deionised water and the range of this calibration curve (from 12.0 to 240.0  $\mu\text{g}/\text{mL}$  for DPM, 12.0 to 240.0  $\mu\text{g}/\text{mL}$  for DBM, 20.0 to 200.0  $\mu\text{g}/\text{mL}$  for PTM, 6.0 to 240.0  $\mu\text{g}/\text{mL}$  for FSM, and 10.0 to 200.0  $\mu\text{g}/\text{mL}$  for APL).

**2.5. Validation of Method.** Method validation of quantitative analysis was performed on parameters such as linearity, precision, recovery, and specificity of the five drugs. The linearity of response was performed using plotting peak areas against the concentration of the injected standard. Method precision was evaluated based on intraday and interday variability. Intraday variability was conducted by injecting the triplicate determination of quality control samples (12.0, 60.0, 120.0, and 240  $\mu\text{g}/\text{mL}$  for DPM and DBM; 6.0, 60.0, 120.0, and 240  $\mu\text{g}/\text{mL}$  for FSM, 20.0, 50.0, 100.0; and 200  $\mu\text{g}/\text{mL}$  for PTM, 10.0, 50.0, 100.0, and 200  $\mu\text{g}/\text{mL}$  for APL) six consecutive times in the same day. Interday variability was realized by using the same quality control samples for six successive days. The relative standard deviation (RSD) values were calculated for the integration area and considered to be the measure of precision. The limit of detection (LOD) for each marker compound was determined at signal-to-noise ratios (S/N) of 3. For limit of quantification (LOQ), the ratio considered was 10:1 with RSD % value less than 10% [30, 31]. Accuracy of the method

was determined by addition of known amounts of DPM, DBM, PTM, FSM, and APL standard drugs ( $n = 3$ , at each level of 50, 100, and 150% levels) into infusion samples in triplicate. In this work, the mean recovery of the DPM, DBM, PTM, FSM, and APL concentration was  $100 \pm 2\%$  for acceptance. The forced degradation studies were performed to prove the specificity of the HPLC method. The DPM, DBM, PTM, FSM, or APL samples were prepared with distilled water and later diluted with distilled water, aqueous hydrochloric acid, aqueous sodium hydroxide, or aqueous hydrogen peroxide solution for 5 h at  $60^\circ\text{C}$ . The chromatogram obtained for the degraded preparation was compared with a chromatogram obtained from the standard curve to determine whether or not any degrading peaks were produced and any changes in concentration, retention time, and peak shape of DPM, DBM, PTM, FSM, and APL. Robustness of the developed method was evaluated with respect to small deliberate alterations in the flow rate ( $1.0 \pm 0.1 \text{ mL}\cdot\text{min}^{-1}$ ), the column temperature ( $30 \pm 1^\circ\text{C}$ ), and the buffer concentration ( $50 \pm 2 \text{ mM}$ ).

**2.6. Application for Pharmaceutical Dosage Forms.** This validated assay was used to quantify the amount of DPM, DBM, PTM, FSM, and APL in their commercially available injection dosage form. For test sample solution, an appropriate volume of the aliquot was transferred into 100 mL volumetric flask and diluted to the mark with diluent to obtain a test solution of DPM (0.12 mg/mL), DBM (0.12 mg/mL), PTM (0.10 mg/mL), FSM (0.12 mg/mL), and APL (0.05 mg/mL), respectively. The solution was filtered through a  $0.45 \mu\text{m}$  membrane filter.

**2.7. Stability of Investigated Drugs in Mixtures.** This study was carried out to mimic as closely as possible a projected routine use of the five cardiovascular agents. Commercially available DPM, DBM, PTM, FSM, or APL were mixed in 50 mL polypropylene syringes of 5% glucose or 0.9% sodium chloride injection at concentrations of 3.0 mg/mL, 3.0 mg/mL, 1.0 mg/mL, 4.0 mg/mL, and 10.0 mg/mL, respectively. All admixtures were investigated at room temperature ( $25^\circ\text{C}$ ) over 48 hours with light protection or light exposure. The stability studies were evaluated in triplicate for each type of infusion media and storage condition. The physical parameters such as color change, presence of turbidity, and precipitate were evaluated qualitatively whenever samples were withdrawn. At each time point, the pH values for each admixture were determined with a precision pH meter (model PHSJ-4F, INESA Scientific Instrument Co., Ltd., Shanghai, China). The concentrations of DPM, DBM, PTM, FSM, or APL were determined by the above described HPLC-DAD method. All samples from each syringe were analyzed in triplicate (total  $n = 3$ ). The initial concentration of the five cardiovascular agents was defined as 100%, and subsequent sample concentrations for the drugs in the mixtures were reported as the percentage of the initial concentration. Drug stability was defined as the remaining 90% of the initial value of each drug.

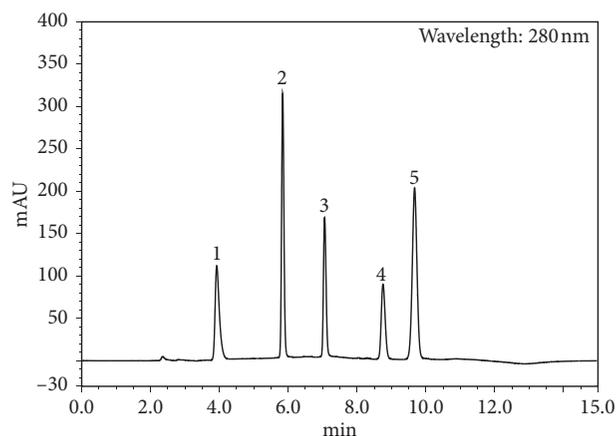


FIGURE 2: HPLC chromatograms of FSM (1), DPM (2), DBM (3), PTM (4), and APL (5).

### 3. Results and Discussion

**3.1. Method Development and Optimization.** The widespread use of a diuretic drug of FSM and diuretic adjuncts such as DPM, DBM, PTM, and APL for the management of congestive heart failure has stimulated our interest to develop a fast and simple analytical method for the simultaneous determination of the five cardiovascular agents in infusion samples and in injection formulations. With regard to the physical and chemical properties of the five cardiovascular agents and the information obtained from the literature, a series of trials of the current method was performed, such as different compositions of mobile phase, detection wavelength, sample preparation procedure, and column lengths, with different pH values and buffering agents. A high-quality separation and symmetric peak shape for all the tested analytes was achieved with mobile phase 50 mM  $\text{KH}_2\text{PO}_4$  and acetonitrile with a simple linear gradient at 1 mL/min flow rate. Under the described conditions, the retention time for DPM, DBM, PTM, FSM, and APL was observed at 3.93, 5.84, 7.06, 8.76, and 9.67 min, respectively, as shown in Figure 2. DPM exhibits a  $\lambda_{\text{max}}$  at 280 nm, DBM  $\lambda_{\text{max}}$  at 271 nm, PTM  $\lambda_{\text{max}}$  at 279 nm, FSM  $\lambda_{\text{max}}$  at 279 nm, and APL  $\lambda_{\text{max}}$  at 276 nm (Figure 3). In order to increase the sensitivity for the analytes and applicability for routine work, a wavelength of 280 nm was selected for detection. The forced degradation peaks are well resolved from the corresponding drug and did not interfere with its determination.

**3.2. Method Validation.** Under the above described experimental conditions, the linearity of the proposed method was investigated by plotting the peak area DPM, DBM, PTM, FSM, and APL versus the concentration of each standard drug. The characteristic parameters for regression equations of the proposed HPLC method are given in Table 1. The values of LOD in the developed method were 0.48, 0.12, 0.20, 0.24, and  $0.18 \mu\text{g}/\text{mL}$  for DPM, DBM, PTM, FSM, and APL, respectively. The calculated LOQ values obtained were 1.80, 0.42, 0.70, 0.90, and  $0.63 \mu\text{g}/\text{mL}$  for DPM, DBM, PTM, FSM, and APL, respectively, with %RSD less than five (accepted criteria in less than 10%).

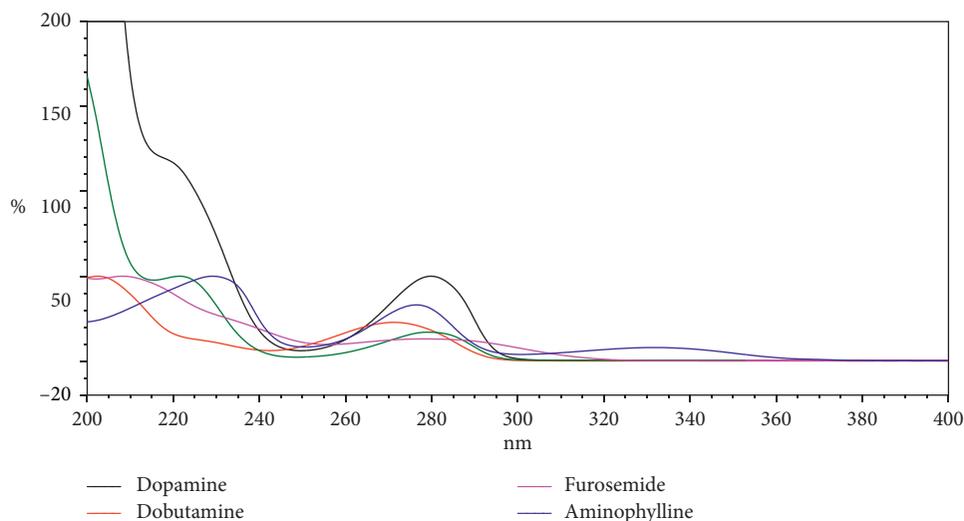


FIGURE 3: Overlaid UV absorption spectrum of FSM, DPM, DBM, PTM, and APL.

TABLE 1: Analytical parameters for DPM, DBM, PTM, FSM, and APL.

Analytical parameter	DPM	DBM	PTM	FSM	APL
$\lambda_{\max}$ wavelength (nm)	280	271	279	279	276
Retention time(min)	3.93	5.84	7.06	8.76	9.67
Theoretical plate (mean $\pm$ SD)	5102 $\pm$ 86	34975 $\pm$ 1595	39586 $\pm$ 1724	27469 $\pm$ 1061	23985 $\pm$ 1362
Linear range (mg/L)	12.0 – 240.0	12.0 – 240.0	20.0 – 200.0	6.0 – 240.0	10.0 – 200.0
Linear equation	$Y = 0.9892X - 2.0961$	$Y = 0.9672X + 0.9111$	$Y = 1.6493X + 1.4782$	$Y = 4.5218X + 32.275$	$Y = 2.9828X - 1.3333$
Coefficient of correlation (r)	0.9999	0.9993	0.9995	0.9990	0.9998
Detection limit (mg/L)	0.48	0.12	0.20	0.24	0.18
Quantification limit (mg/L)	1.80	0.42	0.70	0.90	0.63

The precision and accuracy data for both inter- and intraday analysis of DPM, DBM, PTM, FSM, and APL in the quality control samples at three levels is depicted in Table 2. The results of precision and accuracy were found to be within the acceptable limits and revealed that the new method is precise and accurate. Degradation was observed in different stress conditions, and the peaks for DPM, DBM, PTM, FSM, and APL were clearly separated from peaks for the degradation products. Table 3 represents the assay and purity check for DPM, DBM, PTM, FSM, and APL under different stress conditions. The interference between each compound with degradation products can be accurately located to a certain extent by overall shape or 3D absorption spectra. The peak purity index for the active ingredients and degradants was found to be greater than 0.999, which proves that this method is specific in nature. The robustness results are displayed in Table 4. It was demonstrated that applied minor variations of the flow rate, column temperature, and buffer concentration did not affect the recovery of the five studied drugs.

**3.3. Assay of Pharmaceutical Dosage Forms.** The validated method was applied to the determination of DPM, DBM, PTM, FSM, and APL in the commercially available injection

dosage form. The results are shown in Table 5 and are in good agreement with those obtained using the comparison methods described in the Chinese Pharmacopoeia (2015 edition) [32].

**3.4. Stability of Investigated Drugs in Mixtures.** The mean concentrations over time of DPM, DBM, PTM, FSM, and APL diluted with 5% glucose or 0.9% sodium chloride injection and packaged in polypropylene syringes are outlined in Tables 6 and 7, respectively. Defined as a decomposition of  $\leq 10\%$  compared to the initial concentration, DPM, DBM, PTM, FSM, and APL were stable in either 5% glucose or 0.9% sodium chloride injection and at each storage condition over the whole study period. After 48 hours, there were no visible signs of particulate matter in any of the polypropylene syringes. Each solution remained colorless over the course of the study. There were no major changes in pH for all of the clear solutions. The mean ( $\pm$ standard deviation) pH values were  $3.71 \pm 0.11$ ,  $3.55 \pm 0.38$ ,  $4.70 \pm 0.20$ ,  $9.11 \pm 0.05$ , and  $8.62 \pm 0.06$  for DPM, DBM, PTM, FSM, and APL stored in 5% glucose at  $25^\circ\text{C}$ , respectively. The mean ( $\pm$ standard deviation) pH values were  $4.05 \pm 0.19$ ,  $3.50 \pm 0.29$ ,  $4.50 \pm 0.23$ ,  $9.10 \pm 0.05$ , and  $8.88 \pm 0.02$  for DPM,

TABLE 2: Validation of HPLC method.

Compound	Measured concentrations ( $\mu\text{g/mL}$ )	Accuracy (%)	Precision RSD (%)	
			Intraday	Interday
DPM hydrochloride	60.0	98.8	1.2	1.9
	120.0	102.1	0.7	1.4
	180.0	101.9	1.6	1.8
DBM hydrochloride	60.0	100.4	1.1	1.7
	120.0	100.2	0.3	0.8
	180.0	101.9	1.0	1.4
PTM mesylate	50.0	100.8	0.6	1.2
	100.0	101.3	0.2	1.1
	150.0	99.8	1.4	0.8
FSM	60.0	102.6	1.5	2.3
	120.0	99.4	0.9	0.5
	180.0	102.0	0.7	1.9
APL	50.0	98.5	1.2	1.6
	100.0	101.4	0.6	2.0
	150.0	99.9	0.7	2.5

TABLE 3: Summary of the forced degradation of DPM, DBM, PTM, FSM, and APL.

Compound	Stress condition	Degradation (%)	Peak purity index
DPM hydrochloride	Acidic	3.4	0.9992
	Alkaline	44.6	0.9999
	Oxidative	1.9	1.0000
DBM hydrochloride	Acidic	1.2	0.9998
	Alkaline	51.4	0.9999
	Oxidative	33.7	0.9999
PTM mesylate	Acidic	40.5	0.9998
	Alkaline	42.2	0.9995
	Oxidative	2.3	0.9999
FSM	Acidic	16.1	0.9998
	Alkaline	25.3	0.9999
	Oxidative	7.6	0.9999
APL	Acidic	1.1	1.0000
	Alkaline	4.5	0.9991
	Oxidative	10.6	0.9995

TABLE 4: The robustness of the proposed HPLC method.

Parameter	Variable	Recovery (%)				
		DPM	DBM	PTM	FSM	APL
Flow rate ( $\text{mL}\cdot\text{min}^{-1}$ )	0.9	97.8	98.8	98.4	99.9	98.9
	1.0	97.5	100.6	101.3	98.7	98.4
	1.1	100.3	98.1	98.5	100.1	99.7
	29	99.5	99.1	100.2	99.4	98.8
Column temperature ( $^{\circ}\text{C}$ )	30	99.6	98.9	99.7	98.6	99
	31	98.8	99.2	99.7	100.5	99.4
	48	99.2	99.9	97.9	99.6	101.3
Buffer concentration ( $\text{mM}$ )	50	100.9	98.6	101.6	100.7	98.6
Mean	52	98.7	98.3	98.3	98.9	100.5
%RSD		99.1	99.1	99.5	99.6	99.4
		1.1	0.8	1.3	0.8	1.0

TABLE 5: The results obtained by the proposed methods and the reference methods.

Compound	The proposed methods		The Chinese Pharmacopoeia (2015)	
	Accuracy (%)	RSD (%)	Accuracy (%)	RSD (%)
DPM hydrochloride	100.7	1.1	100.1	0.8
DBM hydrochloride	97.8	1.2	98.7	0.5
PTM mesylate	98.2	0.8	99.4	0.6
FSM	101.6	0.4	99.8	1.1
APL	98.4	1.0	99.2	0.9

TABLE 6: Mean concentrations of DPM, DBM, PTM, FSM, and APL in 0.9% sodium chloride injection with light protection or light exposure at 25°C.

Storage conditions	Time (hours)	DPM	DBM	PTM	FSM	APL
Light protection	0	100.0 ± 1.3	100.0 ± 1.0	100.0 ± 0.6	100.0 ± 0.8	100.0 ± 1.4
	2	102.5 ± 1.9	100.3 ± 0.4	97.6 ± 2.2	100.1 ± 0.3	100.6 ± 0.2
	4	102.5 ± 0.8	99.8 ± 1.2	100.0 ± 0.2	98.7 ± 1.1	98.9 ± 1.1
	8	102.8 ± 0.4	101.7 ± 0.7	99.9 ± 0.4	98.8 ± 0.5	100.7 ± 0.9
	24	103.0 ± 0.5	100.9 ± 0.6	101.9 ± 1.1	99.7 ± 0.2	100.5 ± 0.5
	48	103.2 ± 1.4	99.1 ± 1.2	100.5 ± 0.8	97.8 ± 1.1	98.6 ± 0.4
Light exposure	0	100.0 ± 1.1	100.0 ± 0.4	100.0 ± 0.7	100.0 ± 0.2	100.0 ± 0.6
	2	102.0 ± 0.4	101.2 ± 0.9	100.9 ± 1.0	99.1 ± 0.4	100.7 ± 1.3
	4	102.2 ± 0.5	103.0 ± 2.3	99.8 ± 0.2	99.3 ± 0.7	101.0 ± 1.2
	8	101.5 ± 1.3	100.7 ± 0.8	100.6 ± 0.3	98.1 ± 1.0	97.7 ± 2.0
	24	101.8 ± 0.3	100.2 ± 0.5	97.6 ± 0.2	99.2 ± 0.8	98.9 ± 0.3
	48	102.3 ± 1.8	98.1 ± 1.4	96.2 ± 1.4	98.3 ± 1.3	98.4 ± 1.0

TABLE 7: Mean concentrations of DPM, DBM, PTM, FSM, and APL in 5% glucose injection with light protection or light exposure at 25°C.

Storage conditions	Time (hours)	DPM	DBM	PTM	FSM	APL
Light protection	0	100.0 ± 0.4	100.0 ± 0.2	100.0 ± 1.1	100.0 ± 0.6	100.0 ± 0.5
	2	100.8 ± 1.2	102.1 ± 1.1	99.5 ± 0.3	100.3 ± 0.2	98.4 ± 1.0
	4	101.6 ± 1.3	101.8 ± 0.6	100.2 ± 1.0	103.0 ± 1.8	99.3 ± 0.2
	8	102.0 ± 0.4	100.9 ± 0.6	101.1 ± 1.2	98.8 ± 2.1	98.7 ± 0.8
	24	101.7 ± 1.5	98.6 ± 1.7	98.8 ± 0.8	98.2 ± 0.8	99.1 ± 0.6
	48	101.6 ± 0.8	101.8 ± 1.3	98.7 ± 1.6	100.0 ± 0.3	98.4 ± 1.5
Light exposure	0	100.0 ± 0.5	100.0 ± 1.0	100.0 ± 0.5	100.0 ± 0.2	100.0 ± 0.7
	2	99.3 ± 1.6	101.6 ± 0.9	98.5 ± 1.9	98.0 ± 1.4	98.0 ± 1.1
	4	100.2 ± 0.9	101.9 ± 0.6	100.9 ± 0.2	98.8 ± 0.6	98.4 ± 0.8
	8	102.3 ± 1.1	99.8 ± 1.0	97.4 ± 2.1	99.4 ± 1.5	98.2 ± 0.4
	24	101.2 ± 0.7	101.8 ± 0.5	98.1 ± 1.7	98.9 ± 1.7	99.1 ± 0.2
	48	101.5 ± 1.1	103.3 ± 2.1	96.7 ± 1.0	97.5 ± 1.9	98.9 ± 1.3

DBM, PTM, FSM, and APL stored in 0.9% sodium chloride injection at 25°C, respectively.

#### 4. Conclusion

The present investigation represents a simple and accurate HPLC method for the separation of DPM, DBM, PTM, FSM, and APL. The proposed method could be applied for the analysis of all five drugs in their injection dosage form, infusion samples, and for the evaluation of the stability of investigated drugs in mixtures for endovenous use. On the basis of the results of stability study, DPM, DBM, PTM, FSM, and APL mixed in 5% glucose or 0.9% sodium chloride injection were physically and chemically stable at 25°C for 48 hours (2 days) when stored in polypropylene syringes.

#### Data Availability

All data used to support the findings of this study will be made available from the corresponding author upon reasonable request.

#### Disclosure

Fu-chao Chen and Baoxia Fang are considered as equal first authors.

#### Conflicts of Interest

The authors declare no conflicts of interest.

#### Authors' Contributions

Fu-chao Chen and Baoxia Fang contributed equally to this work

#### Acknowledgments

The authors gratefully acknowledge the financial support for the research: Hubei Province Health and Family Planning Scientific Research Project, China (number: WJ2015MB215) and Technology Key Program of Shiyan, China (number: 18Y73).

#### References

- [1] H. Dokainish, K. Teo, J. Zhu et al., "Heart failure in low-and middle-income countries: background, rationale, and design of the international congestive heart failure study (INTERCHF)," *American Heart Journal*, vol. 170, no. 4, pp. 627–634, 2015.
- [2] P. Kowski, S.-D. Anker, and K.-F. Alhabib, "Heart failure: preventing disease and death worldwide," *ESC Heart Failure*, vol. 1, no. 1, pp. 4–25, 2014.

- [3] H. Shimokawa, M. Miura, K. Nochioka, and Y. Sakata, "Heart failure as a general pandemic in Asia," *European Journal of Heart Failure*, vol. 17, no. 9, pp. 884–892, 2015.
- [4] H. D. Ellison, "Diuretic therapy and resistance in congestive heart failure," *Cardiology*, vol. 96, no. 3-4, pp. 132–143, 2001.
- [5] F. K. Triposkiadis, J. Butler, G. Karayannis et al., "Efficacy and safety of high dose versus low dose furosemide with or without dopamine infusion: the dopamine in acute decompensated heart failure II (DAD-HF II) trial," *International Journal of Cardiology*, vol. 172, no. 1, pp. 115–121, 2014.
- [6] T. Morinaga, "Effect on survival of treatment for heart failure with tolvaptan in addition to low dose furosemide vs. high dose furosemide," *Journal of Cardiac Failure*, vol. 22, no. 9, 2016.
- [7] H.-T. Mebrahtu, I.-V. Borzenets, and H. Zheng, "Clinical experience with low-dose continuous infusion of furosemide in acute heart failure," *Journal of Cardiovascular Pharmacology and Therapeutics*, vol. 17, no. 4, pp. 373–381, 2012.
- [8] K. T. Ng, A. Velayit, D. K. Y. Khoo, A. Mohd Ismail, and M. Mansor, "Continuous infusion versus intermittent bolus injection of furosemide in critically ill patients: a systematic review and meta-analysis," *Journal of Cardiothoracic and Vascular Anesthesia*, vol. 32, no. 5, pp. 2303–2310, 2018.
- [9] A. Kuriyama and S. Urushidani, "Continuous versus intermittent administration of furosemide in acute decompensated heart failure: a systematic review and meta-analysis," *Heart Failure Reviews*, vol. 24, no. 1, pp. 31–39, 2018.
- [10] J. S. K. Chan, T. K. M. Kot, M. Ng, and A. Harky, "Continuous infusion versus intermittent boluses of furosemide in acute heart failure: a systematic review and meta-analysis," *Journal of Cardiac Failure*, vol. 26, no. 9, pp. 786–793, 2020.
- [11] C. J. Vrints, "Focus on acute heart failure: prehospital diagnosis, ultrasound examination and monitoring, pathophysiology, diuretic resistance," *European Heart Journal: Acute Cardiovascular Care*, vol. 7, no. 4, pp. 299–301, 2018.
- [12] A. Ravera, J. M. Ter Maaten, and M. Metra, "Diuretic resistance and chronic heart failure," in *Cardiorenal Syndrome in Heart Failure*, W. Tang, F. Verbrugge, and W. Mullens, Eds., pp. 121–135, Springer, Cham, Switzerland, 2020.
- [13] F. Xing, X. Hu, J. Jiang, Y. Ma, and A. Tang, "A meta-analysis of low-dose dopamine in heart failure," *International Journal of Cardiology*, vol. 222, pp. 1003–1011, 2016.
- [14] M. Bayram, L. De Luca, M. B. Massie, and M. Gheorghide, "Reassessment of dobutamine, dopamine, and milrinone in the management of acute heart failure syndromes," *The American Journal of Cardiology*, vol. 96, no. 6, pp. 47–58, 2005.
- [15] J. N. Nanas, P. Papazoglou, E. P. Tsalgalou et al., "Efficacy and safety of intermittent, long-term, concomitant dobutamine and levosimendan infusions in severe heart failure refractory to dobutamine alone," *The American Journal of Cardiology*, vol. 95, no. 6, pp. 768–771, 2005.
- [16] K. Igarashi and M. Kawahara, "Examination of the cause of changing solution color by mixing aminophylline and dopamine, the compatibility of which was indicated by the supplier," *Yakugaku Zasshi*, vol. 134, no. 2, pp. 293–298, 2014.
- [17] L. Conte, N. R. Pugliese, and A. Giannoni, "Reversal of ticagrelor-induced arrhythmias and Cheyne-Stokes respiration with aminophylline infusion," *Journal of Cardiovascular Pharmacology*, vol. 70, no. 5, pp. 290–292, 2017.
- [18] N. Scroggins, M. Edwards, and R. Delgado, "Increased cost effectiveness with nesiritide vs. milrinone or dobutamine in the treatment of acute decompensated heart failure," *Congestive Heart Failure*, vol. 11, no. 6, pp. 311–314, 2005.
- [19] I.-A. Naguib, E.-A. Abdelaleem, A.-A. Emam, N. W. Ali, and F. F. Abdallah, "Development and validation of HPTLC and green HPLC methods for determination of furosemide, spironolactone and canrenone, in pure forms, tablets and spiked human plasma," *Biomedical Chromatography*, vol. 32, no. 10, 2018.
- [20] S.-R. Paul, L. Kumar, G. Kohli, and A. K. Bansal, "Validated HPLC method for concurrent determination of antipyrine, carbamazepine, furosemide and phenytoin and its application in assessment of drug permeability through Caco-2 cell monolayers," *Scientia Pharmaceutica*, vol. 80, no. 1, pp. 89–100, 2012.
- [21] V. R. Ram, P. N. Dave, and H. S. Joshi, "Development and validation of a stability-indicating HPLC assay method for simultaneous determination of spironolactone and furosemide in tablet formulation," *Journal of Chromatographic Science*, vol. 50, no. 8, pp. 721–726, 2012.
- [22] M. Ahmed, A. Ali, T. Mahmud, M. Qadir, K. Nadeem, and A. Saleem, "Stability-indicating high-performance liquid chromatography method for simultaneous determination of aminophylline and chlorpheniramine maleate in pharmaceutical formulations," *Indian Journal of Pharmaceutical Sciences*, vol. 77, no. 5, p. 515, 2015.
- [23] W. Sila-on, S. Na-Ranong, S. Rakrod, S. Ornlao, and Z. Jounmunkong, "Development and validation of RP-HPLC method for determination of acetazolamide, furosemide and phenytoin extemporaneous suspensions," *Asian Journal of Pharmaceutical Sciences*, vol. 11, no. 1, pp. 138–139, 2016.
- [24] I. Baranowska, P. Markowski, and J. Baranowski, "Development and validation of an HPLC method for the simultaneous analysis of 23 selected drugs belonging to different therapeutic groups in human urine samples," *Analytical Sciences*, vol. 25, no. 11, pp. 1307–1313, 2009.
- [25] N. Patel, M. Taki, P. Tunstell, P. Forsey, and B. Forbes, "Stability of dobutamine 500 mg in 50 ml syringes prepared using a central intravenous additive service," *European Journal of Hospital Pharmacy*, vol. 19, no. 1, pp. 52–56, 2012.
- [26] H. Valizadeh, P. Zakeri-Milani, Z. Islambulchilar, and H. Tajerzadeh, "A simple and rapid high-performance liquid chromatography method for determining furosemide, hydrochlorothiazide, and phenol red: applicability to intestinal permeability studies," *Journal of AOAC International*, vol. 89, no. 1, pp. 88–93, 2006.
- [27] G. E. Benedetto, D. Fico, and A. Pennetta, "A rapid and simple method for the determination of 3, 4-dihydroxyphenylacetic acid, norepinephrine, dopamine, and serotonin in mouse brain homogenate by HPLC with fluorometric detection," *Journal of Pharmaceutical and Biomedical Analysis*, vol. 98, pp. 266–270, 2014.
- [28] C. Yin, C. Tang, and X. Wu, "HPLC determination of aminophylline, methoxyphenamine hydrochloride, noscapine and chlorphenamine maleate in compound dosage forms with an aqueous-organic mobile phase," *Journal of Pharmaceutical and Biomedical Analysis*, vol. 33, no. 1, pp. 39–43, 2003.
- [29] X. Kan, S.-L. Zheng, and C.-Y. Zhou, "UPLC-MS/MS determination of phentolamine in human plasma and its application to a pharmacokinetic study," *Drug Research*, vol. 64, no. 11, pp. 607–612, 2014.
- [30] International Conference on Harmonization (ICH), *Q2 (R1): Validation of Analytical Procedures: Text and Methodology*, ICH, Geneva, Switzerland, 2005.
- [31] H. A. Alhazmi, A. M. Alnami, and M. A. A. Arishi, "A fast and validated reversed-phase HPLC method for simultaneous

determination of simvastatin, atorvastatin, telmisartan and irbesartan in bulk drugs and tablet formulations,” *Scientia Pharmaceutica*, vol. 86, no. 1, pp. 1–13, 2018.

- [32] National Pharmacopoeia Committee, *Pharmacopoeia of the People’s Republic of China, Part 2*, National Pharmacopoeia Committee, Beijing, China, 2015.