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Short Communication

Reversed-phase ion-pair liquid chromatographic method for determining the impurities of furosemide*

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Introduction

The present monograph for furosemide in the European Pharmacopoeia does not include a chromatographic test for related substances, although there is a colorimetric test for the detection of free primary aromatic amines [1]. However, it is considered that the monograph should be strengthened to limit the content of all known potential impurities. The recently published liquid chromatographic method of the United States Pharmacopeia [2] although separating these impurities (Fig. 1), 2-amino-4-chloro-5-sulphamoylbenzoic acid I, 2,4-dichloro-5-sulphamoylbenzoic acid II, 2-chloro-4-(2-furfurylamino)-5 sulphamoylbenzoic acid III and 2,4 bis (2-furfurylamino)-5-sulphamoylbenzoic acid IV from furosemide, suffers from a number of disadvantages. The method requires the use of two impurity standards (I and III) and measurement at two wavelengths of detection which is considered necessary because of the very different absorbance of these impurities. The method also risks the non-detection of polar impurities which may be hidden in the system peak and impurity III which elutes immediately after furosemide. This paper describes a reversed-phase, ion-pair separation of the known potential impurities from furosemide using only one wavelength of detection.

Experimental

Reagents and chemicals

Sodium pentane sulphonate (Interchim, Montluçon, France), cetrimide (Sigma, St Quentin Fallavier, France), glacial acetic acid, acetonitrile, 1-propanol, potassium dihydrogen phosphate, 2-propanol, dioxan, toluene and ammonia were all analytical grade (Merck, Darmstadt, Germany), tetrahydrofuran was HPLC guide (Merck). The impurities (I–IV) were kindly supplied by Hoechst (Frankfurt-am-Main, Germany).

Apparatus

The liquid chromatographic system consisted of a Model 8000 liquid chromatographic pump (Spectra–Physics), a 20 μl fixed volume loop injector (Rheodyne, 7125), a photodiode array detector (Hewlett–Packard 1140 A) and an integrator (Hewlett–Packard 3390 A). The columns used were 250 mm length and 4 mm internal diameter containing 5 μm octyldecylsilyl silica (Nucleosil C-18) for the USP method and octylsilyl silica (Nucleosil C-8, MOS-Hypersil and Lichrosorb RP-8 from Machery & Nagel, Shandon and Merck, respectively) for the proposed method. The mobile phases employed were (a) water–tetrahydrofuran–glacial acetic acid (60:40:1, v/v/v) with a sample diluting solution consisting of sodium pentane

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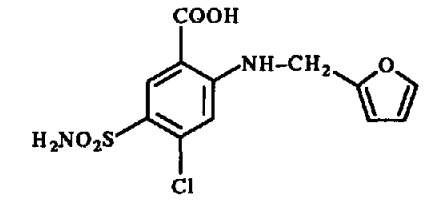
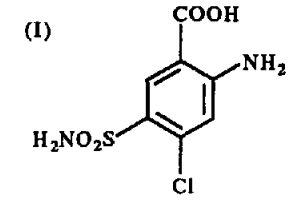


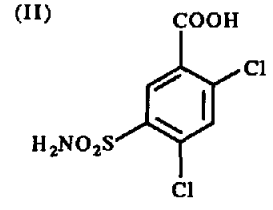
Furosemide
4-Chloro-2-(2-furfurylamino)-5 sulphamoylbenzoic acid

(I)



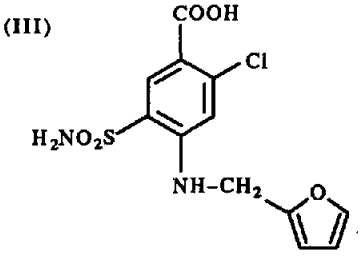
Saluamine
2-Amino-4-chloro-5-sulphamoyl benzoic acid

(II)



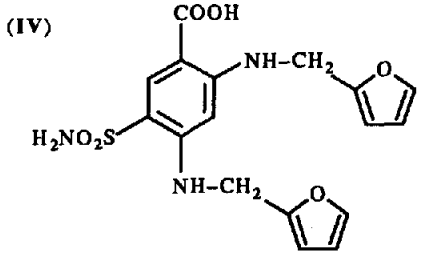
2,4-Dichloro-5-sulphamoyl benzoic acid

(III)



2-Chloro-4-(2-furfurylamino) -5-sulphamoylbenzoic acid

(IV)



2,4-bis(2-furfurylamino) -5-sulphamoylbenzoic acid

Figure 1
Structures of furosemide and its known potential impurities.

sulphonate–glacial acetic acid–water acetonitrile (0.19:2.2:49:49, w/v/v/v) [2] and (b) 0.02 M potassium dihydrogen phosphate–1-propanol–cetrimide (750:300:2.5, v/v/w) and the pH adjusted to 7.0 (with ammonia). The flow rate was 1.0 ml min^{-1} under ambient temperature conditions. Samples for examination were prepared as follows:

Test solution. Dissolve 50 mg of the substance to be examined in the mobile phase and dilute to 50.0 ml with the same solvent.

Reference solution (a). Dissolve 20 mg of 2-chloro-4-(2-furfurylamino)-5-sulphamoylbenzoic acid CRS in the mobile phase and dilute to 20.0 ml with the same solvent.

Reference solution (b). Dilute 1.0 ml of the test solution and 1.0 ml of reference solution (a) to 20.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 20.0 ml with the mobile phase.

Ultraviolet absorbance spectra were recorded in a DU6 UV–vis spectrophotometer (Beckman Instruments).

Thin-layer chromatography was carried out using silica gel GF_{254} plates (laboratory-prepared and precoated (Machery & Nagel, Whatman and Merck)) using as mobile phase a mixture of concentrated ammonia–toluene–dioxan–2-propanol (20:20:30:30, v/v/v/v).

Results and Discussion

It is desirable to revise at the earliest convenience, the European Pharmacopoeia monograph for furosemide to include a satisfactory test for related sustances. A chromatographic method would be preferable.

A thin-layer chromatographic test for the control of the impurities of furosemide had been proposed but was shown not to be entirely satisfactory. There was no adequate separation of all the impurities from furosemide using laboratory prepared plates due to tailing of the furosemide spot, whilst with commercially available pre-coated plates impurity spots in samples of furosemide were more diffuse than the spot of the impurity reference spot thus making comparison difficult. A liquid chromatographic procedure was proposed which had been taken from a previously published paper [3] which described a method capable of determining furosemide in the presence of its degradation products. This had also been shown to separate the known potential impurities (Fig. 2). In the meantime the USP published a method in the first supplement using sodium pentane sulphonate as a counter-ion and requiring the detection at two wavelengths. This is necessary since the absorbances of furosemide and its impurities vary considerably (Table 1, Fig. 3). In this method the impurities eluting before the furosemide peak are controlled at a nominal level of 0.5% using I as an impurity standard and wavelength of detection of 254 nm whilst the peaks eluting after furosemide are controlled at the same nominal level using a solution of III as an impurity standard and a wavelength of detection at 273 nm. The specific absorbance values are given in Table 1

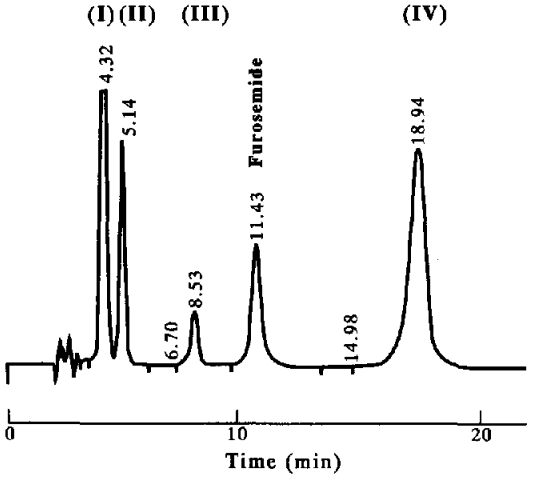


Figure 2
Liquid chromatographic separation of furosemide and its impurities by the proposed method of the European Pharmacopoeia. Column 4 mm × 250 mm Nucleosil C-8, wavelength of detection 238 nm; flow rate 1 ml min^{-1}; chart speed 0.5 ml min^{-1}. The mobile phase consists of 0.02 M potassium dihydrogen phosphate–1-propanol–cetrimide (780:300:2.5, v/v/w) and the pH adjusted to pH 7.0 (with ammonia). Impurities I, II, III and IV as in Fig. 1.

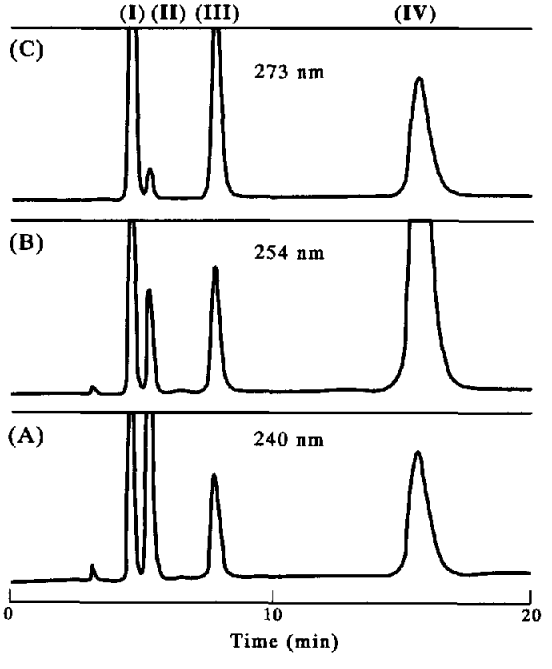


Figure 3
Chromatograms of the impurities of furosemide recorded using different wavelengths of detection. Column 4 mm × 250 mm Nucleosil C 8; flow rate 1 ml min^{-1}; chart speed 0.5 ml min^{-1}. The mobile phase consists of 0.02 M potassium dihydrogen phosphate–1-propanol–cetrimide (780:300:2.5, v/v/w) and the pH adjusted to pH 7.0 (with ammonia). Impurities I, II, III and IV as in Fig. 1.

Table 1
Specific absorbances of furosemide and impurities at various wavelengths and the integration area of the impurities measured at 238 nm after chromtography of a solution containing 0.1%

Substance	Specific absorbance at					Detector response at 238 nm for 0.01% m/V solution
	236 nm	238 nm	240 nm	254 nm	273 nm	
Furosemide	—	—	—	200	630	—
Impurity I	578	378	277	292	650	56.4
Impurity II	423	403	376	120	35	58.0
Impurity III	412	292	219	251	470	43.5
Impurity IV	297	353	420	1378	450	57.8

Impurities I, II, III and IV as in Fig. 1.

from which it can be seen that impurity II will be underestimated. Impurity III may well be hidden or partially hidden in the tail of the furosemide peak. Another disadvantage of this method is that the counter-ion is contained in the solution used to prepare the test and reference solutions and is not present in the mobile phase resulting in the appearance of a system peak in the chromatograms which may hide polar impurities. A typical chromatogram is shown in Fig. 4.

The proposed method using cetrimide as a counter-ion gave good separations between the impurities and furosemide (Table 2) using

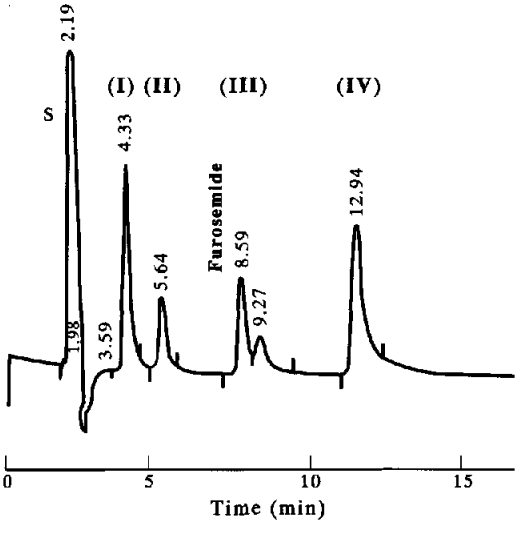


Figure 4
Liquid chromatographic separation of furosemide and its impurities using the chromatographic conditions described in the First Supplement to the USP XXII. Column 4 × 250 mm Nucleosil C-18, wavelength of detection 254 nm; flow rate 1 ml min^{-1}; chart speed 0.5 mm min^{-1}. The diluting solution consists of acetonitrile–water (500:500, v/v) containing 0.96 g sodium pentane sulphonate and 11 ml of glacial acetic acid. The mobile phase consists of water–tetrahydrofuran–glacial acetic acid (600:400:10, v/v/v). S is the system peak and I, II, III and IV are the peaks of the impurities (see Fig. 1).

Table 2
Resolution factors calculated using the proposed chromatographic method

	Nucleosil C8	Lichrosorb RP8	Mos-Hypersil
Rs I/II	2.0	1.7	2.6
Rs II/III	6.9	6.8	4.0
Rs III/F	3.5	4.2	3.4
Rs F/IV	6.3	6.3	6.1

Column performance must ensure that Rs III/F is not less than 3.0, i.e. between 2-chloro-4-*N*-furfurylamino-5-sulphamoylbenzoic acid and furosemide. I, II, III and F as in Fig. 1.

different types of octylsilyl reversed-phase columns. Impurities, I, II and III were eluted before the furosemide peak so that impurity III can be easily integrated and there is no system peak since the mobile phase is employed to prepare the test and reference solutions. Using 254 nm as the wavelength of detection and impurity III to limit the impurities at a level of 0.5% then the area of any peak eluting before the furosemide peak in the chromatogram of the test solution should not exceed the area of the peak in the chromatogram of the reference solution and any peak appearing after the furosemide peak should not exceed 6 times the area of the peak in the chromatogram of the reference solution. However, using this procedure impurity II is underestimated. The differences in responses of the impurities at different wavelengths of detection are shown in Table 1.

It was considered best to employ a wavelength of detection at which the responses of the impurities were similar and it has been shown (Table 1) that at 238 nm all the impurities had a similar absorbance. This was confirmed by the integration values obtained from the chromatogram of a mixture of equal concentration of the impurities. Impurity III being the substance eluting the closest to the

Table 3
Determination of impurities in a number of samples of furosemide by proposed Ph.Eur. method

Impurity	Relative retention*	Percentage impurities found in samples			
		Sample 1	Sample 2	Sample 3	Sample 4
Unknown 'a'	0.19	<0.01	<0.01	<0.01	<0.01
Impurity I	0.38	0.10	0.05	0.03	0.05
Unknown 'b'	0.60	NI	NI	ND	ND
Unknown 'c'	0.65	0.04	ND	ND	ND
Impurity III	0.75	0.12	ND	ND	ND
Unknown 'd'	1.40	0.01	0.01	<0.01	<0.01
Impurity IV	1.70	0.13	0.04	0.04	0.04
Total impurities		0.40	0.10	0.07	0.09

NI, not integrated; ND, not detected.
*Relative retention to furosemide.
Impurities I, III and IV as in Fig. 1.

furosemide peak is considered the most appropriate substance to employ in a reference solution to confirm the performance of the column employed (minimum resolution requirement of 3 for the separation of impurity III and furosemide) and also to limit each impurity to a level of 0.25% and a total impurity level of 0.5%. Using this method and impurity III as the standard the recovery of impurity IV from a 'spiked' sample (at the 0.25% level) was shown to be 97%. Examination of a number of samples of furosemide has shown that the limits proposed above would be reasonable since all of the samples examined would comply (Table 3).

Conclusion

It appears that the proposed method is superior to the existing method for the limitation of impurities in furosemide. Currently this method is under study by collaborative trial and it is hoped to publish this method in a revised European Pharmacopoeia monograph for furosemide.

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