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Short communication

Development and validation of RP-HPLC method for the determination of genotoxic alkyl benzenesulfonates in amlodipine besylate

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ABSTRACT

The present paper describes a simple isocratic reverse phase HPLC method for the determination of four genotoxic alkyl benzenesulfonates (ABSs) viz. methyl, ethyl, *n*-propyl and *iso*propyl benzenesulfonates (MBS, EBS, NPBS and IPBS) in amlodipine besylate (ADB). Good resolution between benzene sulfonic acid (BSA), MBS, EBS, NPBS, IPBS and ADB was achieved with Inertsil ODS 3V (150 mm × 4.6 mm, 5 μm) column using a 65:35 (v/v) mixture of 1% triethyl amine, pH adjusted to 3.0 with orthophosphoric acid and acetonitrile as mobile phase. The flow rate was 1.0 ml/min and the elution was monitored at 220 nm. The factors involved in the method development are discussed. This method was validated as per International Conference on Harmonization (ICH) guidelines and is able to quantitate MBS, EBS, NPBS and IPBS at 21, 32, 35 and 28 ppm levels, respectively with respect to 5 mg/ml of ADB. The method is linear in range of 75–180 ppm of ABSs, which matches the range of 50–120% of estimated permitted level (150 ppm) of ABSs. ABSs were not present in the three studied pure and tablet batches of ADB.

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1. Introduction

Regulatory issues related to the presence of genotoxic impurities have arisen with a greater frequency due to enhanced technological capability in identifying impurities and increased focus on their potential impact on human health [1]. As per the guideline from the European Medicines Agency on the limits of genotoxic impurities, a threshold of toxicological concern (TTC) value of 1.5 μg/day intake of a genotoxic impurity is considered to be associated with an acceptable risk for most of the pharmaceuticals. The concentration limit of permitted genotoxic impurity in ppm is the ratio of TTC in microgram/day and dose in gram/day [2].

Amlodipine besylate (ADB), chemically described as 3-ethyl-5-methyl-(4RS)-2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate benzenesulfonate is an anti hypertensive agent [3–6]. Since, benzene sulfonic acid (BSA) or besylate is the counter ion of ADB, the usage of methyl, ethyl, *n*-propyl or *iso*propyl alcohol either at any stage of synthesis or crystallization, leads to the formation of genotoxic methyl, ethyl, *n*-propyl and *iso*propyl benzenesulfonates (MBS, EBS, NPBS and IPBS), respectively (Scheme 1) [7]. ADB is administered at a maximum daily dose of 10 mg/day [8] in the form of tablets (2.5, 5.0, 10.0 mg with trade name as Amlo-H). Hence, the estimated permitted level of ABSs (alkyl benzenesulfonates) in ADB is 150 ppm/day. In literature, two GC–MS [9,10] and one LC–MS methods [11] were found for the determination of ABSs. Recently, D.P. Elder et al., reviewed about the control and analysis of alkyl esters of alkyl and aryl sulfonic acids in novel active pharmaceutical ingredients [12]. However, all the reported methods involve highly sophisticated instruments, which are not generally available in most of the up coming pharmaceutical industries and no report is concentrated on the analysis of ABSs in ADB. Therefore, in continuation to our previous publication on the determination of genotoxic alkyl methanesulfonates in a drug substance [13], we have developed a simple isocratic RP-HPLC method that can quantitate at permitted levels of ABSs in ADB. This method is validated as per ICH guidelines [14] in terms of limit of detection (LOD), limit of quantification (LOQ), linearity, precision, accuracy, specificity and robustness.

2. Experimental

2.1. Chemicals

All chemicals and solvents were of analytical grade. Triethyl amine and acetonitrile (HPLC grade) were procured from Merck, India. Methyl benzenesulfonate and ethyl benzenesulfonate were purchased from Sigma–Aldrich Corporation, Bangalore, India.

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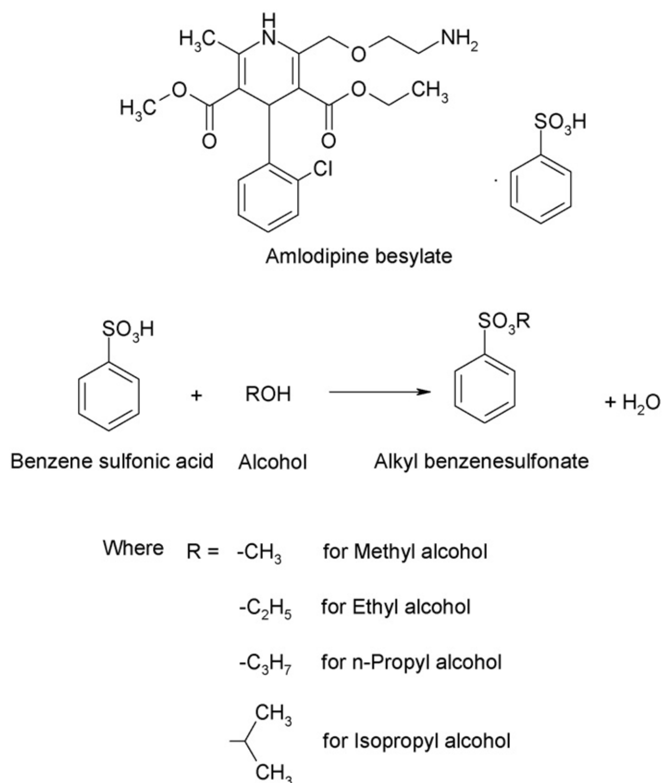

Scheme 1. Formation of alkyl benzenesulfonates from amlodipine besylate.

*Iso*propyl benzenesulfonate was purchased from Shanghai Synnad Chemical Co, Ltd., Shanghai, China. *n*-Propyl benzenesulfonate and pure amlodipine besylate were obtained from our R & D division. ADB tablets (Code: Amlo-H) were obtained from the formulation division of Hetero Drugs Ltd., Hyderabad, India.

2.2. Chromatographic conditions

Analysis was carried out on Waters Alliance 2695 separation module equipped with 2996 PDA detector (Waters Corporation, Milford, USA). However, for convenience, the elution was monitored at 220 nm. Separation was achieved by using Inertsil ODS 3 V column (150 mm × 4.6 mm, 5 μm, GL Sciences Inc., Japan) with mobile phase containing a 65:35 (v/v) mixture of 1% triethyl amine, pH adjusted to 3.0 with orthophosphoric acid and acetonitrile. The flow rate of the mobile phase was kept at 1.0 ml/min. Acetonitrile was used as diluent. Twenty microliters of sample solution is injected each time.

2.3. Sample preparation

One mg/ml individual stock solutions of MBS, EBS, NPBS, IPBS were prepared by dissolving 10 mg each of ABSs separately in 10 ml of acetonitrile. These solutions were used for identifying retention times. Three mg each of alkyl benzenesulfonates and ADB dissolved in 10 ml of diluent was used for resolution studies (Fig. 1). For validation studies, required volumes of MBS, EBS, NPBS, IPBS stock solutions and 50 mg of ADB were taken in 10 ml volumetric flask. The contents were dissolved and diluted up to the mark with acetonitrile. Since ADB and all the four ABSs are soluble in acetonitrile, tablet powder equivalent to 50 mg of ADB and required volumes of MBS, EBS, NPBS, IPBS stock solutions were dissolved in 10 ml of acetonitrile, sonicated well and the filtered solution was used for analysis.

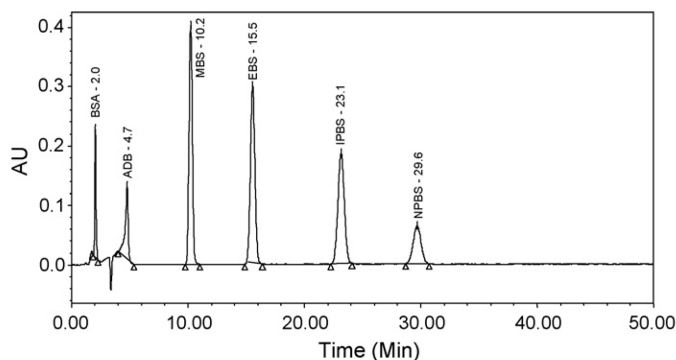

Fig. 1. LC chromatogram of 0.3 mg/ml each of ADB and alkyl benzenesulfonates.

3. Results and discussion

3.1. Method development

The present method was developed by altering stationary and mobile phases sequentially and observing their influence on the resolutions of all the four ABSs, BSA and ADB. As a preliminary investigation, Waters Symmetry C18 (150 mm × 4.6 mm × 5 μm) column was selected as appropriate stationary phase. Since, all the four ABSs were derived from benzenesulfonic acid, the investigation is commenced with a buffer at acidic pH. Hence, 0.1% formic acid was chosen as an aqueous solution. Since, most of the compounds under study have wavelength maximum as 220 nm, in order to avoid blank interference, acetonitrile was chosen as organic solvent because of its low wavelength maximum (190 nm) when compared to methanol (205 nm). The buffer and acetonitrile ratio was fixed as 75:25 (v/v). All the compounds were eluted, but the resolution between MBS and NPBS is very low. Now, in order to get a good resolution, the length of the column was increased and thus Kromasil C18 (250 mm × 4.6 mm × 5 μm) column was opted with the same mobile phase, but no improvement in the resolution was observed. Then, it was planned to change mobile phase and hence, 0.02 M sodium dihydrogen phosphate aqueous solution, pH adjusted to 5.4 with 1 M sodium hydroxide solution in combination with acetonitrile in 60:40 (v/v) ratio was used with above column. Good resolution for all the compounds were achieved but blank interference in EBS peak was observed. Since, EBS is to be quantified at a low level, blank interference was considered as a major problem. Now, 1% triethyl amine in water, pH adjusted to 4.0 with orthophosphoric acid with acetonitrile in 75:25 (v/v) ratio was selected. However, Inertsil ODS 3 V (250 mm × 4.6 mm × 5 μm) column was selected because of its appreciable carbon loading. In this condition, good separation was achieved for all the compounds with out any blank interference. However, the problem was with longer retention times. Finally, a short column (Inertsil ODS 3V, 150 mm × 4.6 mm × 5 μm) was selected. pH of the buffer was

Table 1
LOD, LOQ and linear regression analysis and precision data

Parameter	MBS	EBS	NBPS	IPBS
LOD (ppm)[a]	7	11	12	9
LOQ (ppm)[a]	21	32	35	28
Linear range (ppm)[a]	75–180	75–180	75–180	75–180
Slope	835	945	687	993
Intercept	331	678	898	915
Correlation coefficient	0.9999	0.9998	0.9997	0.9999
Precision (% R.S.D.)[b]	0.43	0.98	0.63	0.87
Intermediate Precision (% R.S.D.)[b]	1.52	1.81	1.92	1.12

[a] LOD, LOQ and linear ranges are given in ppm with respect to 5 mg/ml of ADB.
[b] Six determinations using 150 ppm of ABS with respect to 5 mg/ml of ADB.

Table 2
Evaluation of accuracy and specificity of the proposed method

Sample	% Recovery of MBS[a] (mean ± % R.S.D.)			% Recovery of EBS* (mean ± % R.S.D.)		
	75 ppm	150 ppm	180 ppm	75 ppm	150 ppm	180 ppm
Pure sample-1	98.5 ± 0.26	99.3 ± 0.45	98.1 ± 0.33	99.5 ± 0.56	99.5 ± 0.33	99.7 ± 0.66
Pure sample-2	99.6 ± 0.76	99.2 ± 0.12	98.3 ± 0.78	98.5 ± 0.45	99.6 ± 0.65	98.7 ± 0.78
Pure sample-3	98.3 ± 0.79	99.5 ± 0.12	98.7 ± 0.90	99. 6 ± 0.65	99.1 ± 0.76	98.3 ± 0.56
Tablet-1	99.8 ± 1.11	98.3 ± 1.23	98.5 ± 1.30	98.7 ± 1.21	98.9 ± 1.13	99.8 ± 1.32
Tablet-2	99.1 ± 1.32	98.6 ± 1.24	99.9 ± 1.65	98.1 ± 1.23	99.4 ± 1.08	99.9 ± 1.77
Tablet-3	99.7 ± 1.51	98.7 ± 1.56	99.3 ± 1.67	99.8 ± 1.13	98.6 ± 1.89	98.8 ± 1.74

Sample	% Recovery of NPBS[a] (mean ± % R.S.D.)			% Recovery of IPBS[a] (mean ± % R.S.D.)		
	75 ppm	150 ppm	180 ppm	75 ppm	150 ppm	180 ppm
Pure sample-1	99.5 ± 0.85	98.8 ± 0.34	99.6 ± 0.55	98.5 ± 0.61	99.1 ± 0.12	99.6 ± 0.76
Pure sample-2	99.6 ± 0.41	98.8 ± 0.52	99.4 ± 0.23	98.7 ± 0.65	99.3 ± 0.67	99.2 ± 0.15
Pure sample-3	99.7 ± 0.15	99.2 ± 0.77	98.4 ± 0.24	99.8 ± 0.43	99.7 ± 0.34	99.4 ± 0.22
Tablet-1	99.8 ± 1.54	98.6 ± 1.09	98.8 ± 1.32	99.6 ± 1.99	99.93 ± 1.38	98.6 ± 1.00
Tablet-2	99.1 ± 1.66	99.2 ± 1.00	99.8 ± 1.80	99.8 ± 1.45	98.4 ± 1.64	98.1 ± 1.38
Tablet-3	98.6 ± 1.28	98.8 ± 1.29	98.4 ± 1.11	98.7 ± 01.34	99.2 ± 1.67	98.6 ± 1.90

[a] Mean value of three determinations.

adjusted to 3.0 with orthophosphoric acid and mobile phase ratio was maintained in 65:35 ratio. All the ABSs, BSA and ADB were eluted at lower retention times with appropriate resolutions. Peak shapes of all the alkyl benzenesulfonates were good. Even though a small distortion of ADB peak was observed, it was not considered because the quantitation is mainly concentrated on ABSs but not on ADB. Room temperature was found to be adequate for the analysis.

3.2. Method validation

The method validation was started by injecting 150 ppm individual solutions of ABSs with respect to 5 mg/ml of ADB and determining their S/N (signal to noise) ratios. Now, to determine LOD and LOQ values, ABSs concentrations were reduced sequentially such that they yield S/N ratio as 3 and 10, respectively. The determined LOD and LOQ values were presented in Table 1. Linearity for all the four ABSs was fixed in the range of 50–120% of the estimated permitted level (viz.150 ppm with respect to 5 mg/ml of ADB solution). Hence, 75, 100, 120, 150, 180 ppm solutions of four ABSs were prepared and injected individually. The calibration curves were drawn between the peak areas versus concentration of ABSs. The slope, intercept and correlation coefficient values were derived from liner least-square regression analysis and the data presented in Table 1 reveals that an excellent correlation existed between the peak areas and the concentrations of ABSs. The precision was evaluated at two levels viz. repeatability and intermediate precision. Repeatability was checked by calculating the relative standard deviation (% R.S.D.) of six replicate determinations by injecting six freshly prepared solutions containing 150 ppm of ABSs on the same day. The same experiments were done on six different days for evaluating intermediate precision. The low % R.S.D. values confirm the good precision of the developed method (Table 1). When three pure and tablet solutions of 5 mg/ml ADB were injected, ABSs were not at all detected in them. Hence, recovery studies by the standard addition method were performed to evaluate accuracy and specificity. Accordingly, the accuracy of the method was determined by spiking 75, 150, 180 ppm of ABSs separately to three batches of pure ADB (5 mg/ml). Each determination was carried three times. The recovery data indicates the accuracy of the method (Table 2). The specificity defined as the ability of method to measure the analyte specifically in the sample matrix, was determined by analyzing the tablets of ADB. The recovery studies were performed by spiking 75, 150, 180 ppm of ABSs separately to three different batch tablets of ADB (5 mg/ml). The recovery data is presented in Table 2 reveals that no extra amount of ABSs than spiked was found in pure and tablets powders of ADB. It was also observed that the common excipients used in the tablets were not interfering at the retention times of ABSs and ADB. In addition, the peak purities of all the alkyl benzenesulfonates were studied individually with PDA detector and found to be homogeneous with no detectable impurities embedded in them. Hence, It was concluded that ABSs were not present in the three studied batches of pure and tablet formulations of ADB. The robustness of the method was studied with deliberate modifications in flow rate of the mobile phase and column temperature. The flow rate of the mobile phase was 1.0 ml/min and the same was altered by 0.2 units i.e. from 0.8 to 1.2 ml/min. The effect of column temperature on resolution was studied at 25 and 32 °C instead of room temperature (28 °C). The results revealed that these changes do not have any impact on chromatographic performance. However, the mobile phase components were held constant as described above. Stability of ABSs in diluent was checked by keeping them in an auto sampler and observing the variations in their peak areas. Methanol was initially tried as diluent in sample preparation. It was observed that NPBS is getting degraded in methanol with in a short time. Hence, acetonitrile was chosen for this purpose and found that MBS, EBS, NPBS and IPBS were stable up to 9, 9, 8 and 6 h, respectively. Therefore, it is recommended to complete ABSs mixture analysis before 6 h of its preparation in acetonitrile.

4. Conclusions

The desired goal of this study is to develop a simple analytical method that is capable of quantifying four unexpected alkyl benzenesulfonates in amlodipine besylate. Hence, a simple isocratic RP-HPLC method, which is able to quantify them at ppm level is developed and validated. The information presented here could be very useful for monitoring of alkyl benzenesulfonates in ADB in its pure and tablet forms.

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