Journal of Pharmaceutical and Biomedical Analysis 50 (2009) 1065–1069

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Short communication

Validation of a sensitive ion chromatography method for determination of monoethylsulfate in Indinavir sulfate drug substance

S. John Prasanna[a,b], Hemant Kumar Sharma[a,*], K. Mukkanti[b], M. Sivakumaran[a], K.S.R. Pavan Kumar[a], V. Jagadeesh Kumar[a]

[a] Aurobindo Pharma Limited, Research Centre, 313, Bachupally, Qutubullapur Mandal, Hyderabad 500 090, India
[b] Centre for Pharmaceutical Sciences, Jawaharlal Nehru Technology University, Kukatpally, Hyderabad 500 085, India

ARTICLE INFO

Article history:
Received 31 October 2008
Received in revised form 12 June 2009
Accepted 20 June 2009
Available online 27 June 2009

Keywords:
Ion chromatography
Monoethylsulfate
Indinavir sulfate

ABSTRACT

The present study relates to the optimization of an ion chromatography method to determine the content of monoethylsulfate at very low levels in Indinavir sulfate drug substance, and subsequent validation of the method to prove its suitability, reliability and sensitivity. Monoethylsulfate is a potential impurity of Indinavir sulfate, and may forms during the preparation as well as during storage. The ion chromatography method was developed in such a way that to enhance the detection level by introducing suppressor, and minimizing acquisition time by using suitable buffer of 3.2 mmole of sodium carbonate and 1 mmole of sodium hydrogen carbonate in water as eluent. The retention time of monoethylsulfate was about 9.5 min and the total acquisition time was 25 min. The optimized method was validated to prove its performance characteristics by demonstrating selectivity, sensitivity (limit of detection and quantification), linearity, precision and accuracy. The established limit of detection and quantification of monoethylsulfate in Indinavir sulfate by this method was found to be 24 ng/ml and 74 ng/ml respectively, and the overall percent accuracy (recovery) of samples evaluated at different concentration levels was found to be 97.1, indicating the sensitivity and accuracy of this optimized ion chromatography method.

1. Introduction

Indinavir sulfate (as ethanol solvate), active ingredient of CRIXIVAN, is a specific and potential inhibitor of HIV-1 protease, and is widely used in the treatment of AIDS. Synthesis of Indinavir base has been described in literature [1]. Indinavir sulfate as crystalline ethanolate is then prepared by dissolving free base in anhydrous ethanol and treated with sulfuric acid in anhydrous ethanol. The reaction scheme is shown in Fig. 1. As reported, at ambient temperature, mixture of primary or secondary alcohols and sulfuric acid react to give monoalkylsulfate esters [2]. Therefore, the process for the treatment of Indinavir free base in anhydrous ethanol with sulfuric acid is carried out under controlled temperature of less than 0 °C to avoid the formation of monoethylsulfate during the preparation. However, it is very important for any drug substance manufacturer to monitor the level of anticipated process related and degradation impurities before commercial release to prove the consistency of the manufacturing process employed, by using appropriate analytical techniques. In addition, prolonged exposure to moisture results in loss of crystalline nature of Indinavir sulfate ethanolate salt, and leads to amorphous Indinavir sulfate, which could end up with free ethanol and sulfuric acid, and may lead to further esterification. This esterification reaction is also quite possible during the storage [3,4]. Therefore, monoethylsulfate is observed to be a potential degradation impurity of Indinavir sulfate, and to be monitored during stability storage as well. It is more dependable to be noted that, any impurity other than active moiety are to be controlled with suitable limits in the drug substance irrespective of its harmful nature as per ICH guidelines on impurities [5]. To the best of our knowledge, there is no toxicity data available for monoethylsulfate, however it has been reported [6] that it is prone to mutagen in *Escherichia coli*.

Subsequently, an ion chromatography (IC) method was optimized to determine the content of monoethylsulfate in Indinavir sulfate as it is a conventional analytical technique for the separation and subsequent determination of inorganic anions and cations as well as organic acids and bases. Monoethylsulfate, the analyte of interest exists as anion in aqueous solution, and the separation was influenced by selecting suitable eluent and stationary phase (packed with quaternary ammonium groups supported by polyvinyl alcohol) and by using conductimetric detection. A suppressor as sulfuric acid was used to reduce the background conductance of the eluent in order to enhance the detectability of monoethylsulfate ions.

* Corresponding author. Tel.: +91 40 23040261; fax: +91 40 23042932.
E-mail address: hemant@aurobindo.com (H.K. Sharma).

0731-7085/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2009.06.041

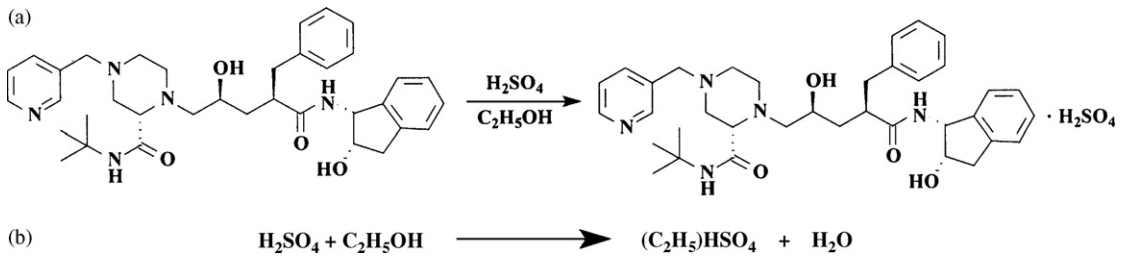

Fig. 1. Reaction scheme: (a) conversion of Indinavir base to Indinavir sulfate. (b) Reaction of sulfuric acid and ethanol to form monoethylsulfate.

It may be noted that, the determination of monoethylsulfate by non-aqueous titration (titrated against tetrabutylammonium hydroxide using pyridine as a solvent) [7], by capillary electrophoresis in indirect UV mode [8] have been reported in literature and also determination of alkylsulfonate and sulfate in atmospheric air were reported [9,10]. The limit of detection and quantification of monoethylsulfate by capillary electrophoresis were found to be 1400 ng/ml and 2800 ng/ml respectively. Nevertheless, the titrimetry method was found to be less accurate and time consuming, whereas capillary electrophoresis method was less sensitive.

The optimized IC method was validated according to ICH guidelines [11] to prove its suitability and reliability for the determination of monoethylsulfate in Indinavir sulfate drug substance during routine as well as stability storage analysis.

2. Materials and methods

2.1. Chemicals and reagents

Potassium salt of monoethylsulfate was prepared at Aurobindo Pharma Ltd., Research Centre, India, and was characterized, used as reference standard. The purity of reference standard sample was 97.40% evaluated by IC method and water content was 0.20% by Karl Fischer titration. The potency of reference standard was assign to be 97.20%. Benzoic acid, cetryltetrabutylammonium bromide (CTAB), methanol, sodium hydroxide and sulfuric acid were supplied by E.Merck India. Water was distilled and purified with Millipore system (Millipore corporation, India). The known related substances of Indinavir sulfate such as (1S,2R)-(−)*cis*-amino-2-hydroxyindane, (2R,4S)-2-benzyl-5-[(2S)-2-[(1,1-dimethylethyl) carbamoyl]piperazin-1-yl]-4-hydroxy-N-[(1S,2R)-2-hydroxy-2,3-dihydro-1H-1-inden-1-yl]pentanamide, (2R,4R)-2-benzyl-5-[(2S)-2-[(1,1-dimethylethyl)carbamoyl]-4-(3-pyrid ylmethyl)piperazin-1-yl]-4-hydroxy-N-[(1S,2R)-2-hydroxy-2,3-dihydro-1H-1-inden-1-yl] pentanamide, 5-[(2S)-2-[(1,1-dimethylethyl)carbamoyl]-4-(3-pyridylmethyl)piperazin-1-yl]-3-benzyltetrahydrofuran-2-one, (2R,4S)-2-benzyl-5-[(2S)-2-[(1,1-dimethylethyl) carbamoyl]-4-[(2R,4S)-2-benzyl-4-hydroxy-N-[(1S,2R)-2-hydroxy-2,3-dihydro-1H-inden-1-yl]pentanamide-5-yl]piperazinyl-1-yl]-4-hydroxy-N-[(1S,2R)-2-hydroxy-2,3-dihydro-1H-inden-1-yl]pentanamide were prepared at Aurobindo Pharma Ltd. Research Centre, India and were used during the study.

2.2. Sample preparation

2.2.1. Standard solution

The standard stock solution of Potassium monoethylsulfate was prepared by weighing accurately about 68 mg of the potassium monoethylsulfate into 50 ml volumetric flask and diluted to volume with water, which is equivalent to 1000 μg/ml of monoethylsulfate. The standard solution containing monoethylsulfate of 1 μg/ml was obtained by diluting the stock solution with appropriate volume of water, and filtered through a 0.45 μm membrane filter.

2.2.2. Test solution

About 100 mg of Indinavir sulfate drug substance was accurately weighed and transferred into a 100 ml volumetric flask, and dissolved the substance completely by using 50 ml of water and diluted to volume with water. This solution was filtered through a 0.45 μm membrane filter.

2.2.3. Mobile phase solution

The mobile phase was prepared by dissolving about 339.2 mg of sodium carbonate and 84 mg of sodium hydrogen carbonate in 1000 ml of water, and the solution was subjected to sonication about 10 min to remove any air bubbles, and filtered through a 0.45 μm membrane filter.

2.2.4. Suppressor solution

50 mmole of sulfuric acid was prepared and used as suppressor regenerating solution.

2.3. Instrumentation

2.3.1. Ion chromatography

An IC system was Metrohm 761 Compact IC consists of conductometric detector and suppressor with peristatic pump equipped with Metrohm 750 Auto sampler. The data handling system was Metrohm 761 Compact IC software. An analytical column, Metrosep A Supp5 (Metrohm, 250 mm × 4.0 mm 5 μm particle size) packed with polyvinyl alcohol with quaternary ammonium groups was used as stationary phase.

2.3.2. Capillary electrophoresis

An Agilent instrument CE system equipped with a diode array detector along with chemstation software for data acquisition and processing was used. Separation was carried out in fused silica capillary with extended light path length (Agilent, Germany) with effective length of 56 cm and i.d. of 50 μm.

2.4. Procedure

2.4.1. Ion chromatography

The detector was operated in conductivity mode and suppressor was in anion self-regenerating suppressors ion recycle mode. The analog range of the detector was set at 50 μS/cm. The instrument parameters were set as mentioned below during the analysis. The column was maintained at ambient temperature. Mobile phase was pumped through the column at a flow rate of 0.7 ml/min. The injection volume was 20 μl. The retention time of the monoethylsulfate was about 9.5 min. The total acquisition time of the analysis was up to 25 min.

2.4.2. Capillary electrophoresis

The background electrolyte consists of 2.5 mmole of benzoic acid, 0.25 mmole of CTAB and 3% of methanol and adjusted the pH to 6.0. The sample and standard were introduced with hydrodynamic

pressure of 50 mbar for 5 s, and the separation was carried out with constant applied voltage of (−)20 kV and temperature at 30 °C. Before introducing sample, the capillary was conditioned with background electrolyte for 3 min. The analyte signal was detected by indirect UV photometric method, the wavelength was set at 350 nm against reference signal at 225 nm. New capillaries were rinsed with sodium hydroxide (4.2 mg/ml) in water for 5 min and with water for 5 min and followed by background electrolyte for 15 min.

3. Results and discussion

3.1. Method development

Monoethylsulfate, an impurity of Indinavir sulfate is available as anionic species in aqueous solution, and is inert to UV photometric absorption. Therefore, it is difficult to determine the content of monoethylsulfate by using conventional analytical techniques like HPLC, where UV photometric detection is employed. However, this kind of anionic or cationic species can be determined either by Ion chromatography or by CE method.

This ion chromatography method was optimized to use 3.2 mmole of sodium carbonate and 1 mmole of sodium hydrogen carbonate as eluent with conductimetric detection. The buffer concentration and the competing ions, the critical factors that influencing the retention time were suitably optimized to achieve the shorter separation time for monoethylsulfate. The suppressor as sulfuric acid was used to enhance the sensitivity of the detection level.

This optimized IC method to determine the content of monoethylsulfate in Indinavir sulfate was validated according to ICH guidelines [11] to evaluate its performance characteristics.

3.2. Validation

The experiments that have been demonstrated during validation studies were selectivity, sensitivity by means of limit of detection and quantification, linearity, precision (system precision, method precision and intermediate precision), stability of sample solution and accuracy, and the results obtained from the experiments were briefly summarized below.

3.2.1. Selectivity

The solution of blank, monoethylsulfate, Indinavir sulfate were prepared separately, and injected as per procedure to identify the retention time of components of sample matrix. And also the sample solution spiked with monoethylsulfate and sample solution spiked with monoethylsulfate along with other known impurities of Indinavir sulfate were prepared peak, and injected as per procedure to confirm any co-elution of peaks due to sample matrix. The chromatograms obtained from the analyses show that, the monoethylsulfate peak was well resolved from background noise and from that of blank, sulfate ion and other components of sample matrix as well, indicating the selectivity of the method to determine the content of monoethylsulfate in Indinavir sulfate. An overlay chromatogram of blank solution, standard solution and sample solution spiked with other known related substances of Indinavir sulfate are shown in Fig. 2.

3.2.2. Sensitivity

The limit of detection (LOD) and limit of quantification (LOQ) were predicted using slope (S) and residual standard deviation (SD) that obtained from a linear regression line performed by using monoethylsulfate solution prepared at lower concentration levels between 100 ng/ml and 1000 ng/ml, is being one of the three approaches described in ICH guidelines [11] for the prediction of

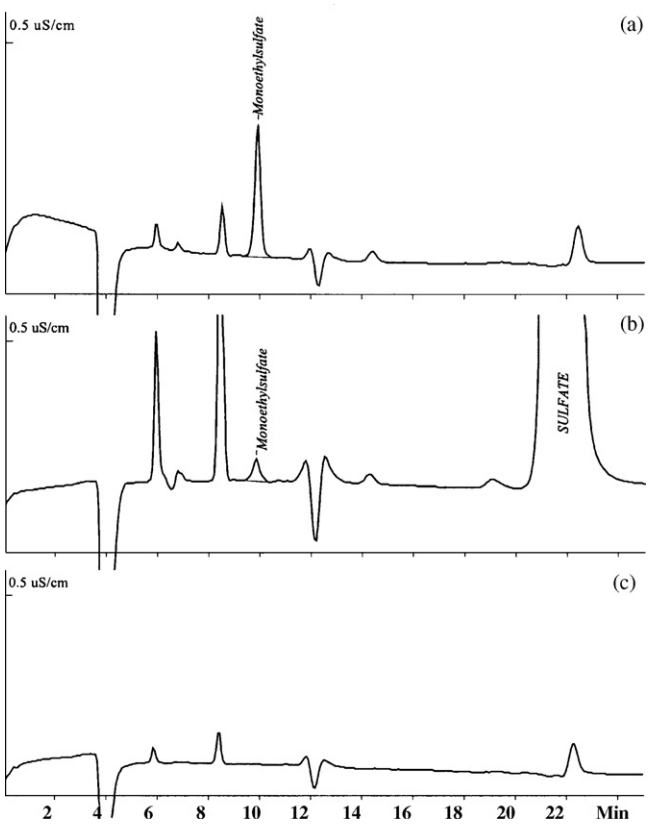

Fig. 2. (a) 1000 ng/ml of standard monoethylsulfate solution, (b) sample solution spiked with the known related compounds of Indinavir sulfate and (c) blank solution.

LOD and LOQ. The formula used for the prediction of LOD and LOQ were 3.3 × SD/S and 10 × SD/S respectively.

The predicted LOD and LOQ levels were found to be 24 ng/ml and 74 ng/ml respectively. The solutions were prepared at the predicted concentration of LOD and LOQ levels, and analyzed for six times, and the percentage relative standard deviation was found to be 3.6 and 2.4 respectively. Thus, the LOD and LOQ values were established to determine the content of monoethylsulfate in Indinavir sulfate.

3.2.3. Linearity

The linear relationship of analyte response against concentration was verified in the working concentration range by analyzing different level of solutions containing monoethylsulfate from about LOQ level (75 ng/ml) to 1200 ng/ml. The linear regression line was plotted against analyte response versus concentration. The correlation coefficient of the regression line was found to be 0.9997. The statistical analysis of linear regression line was evaluated and is summarized in Table 1.

Table 1
The statistical analysis of linearity data.

Statistical parameter	Results
Correlation coefficient (r^2)	0.9997
Concentration range (ng/ml)	Between 75 and 1200
Intercept (a)	0.0362
Slope (b)	0.0038
Standard deviation (intercept)	0.0116
Standard deviation (slope)	1.02×10^{-4}
Standard error estimate (residual standard deviation)	0.0271
Limit of detection (ng/ml)	24 (24)
Limit of quantification (ng/ml)	73 (74)

Parenthesis values are actual LOD and LOQ determined from sensitivity experiment and precision shown at this predicted level.

Table 2
The Comparison of method precision and intermediate precision.

Sample	Monoethylsulfate content (μg/g)	
	Method precision	Intermediate precision
1	254	256
2	286	238
3	259	239
4	267	259
5	264	270
6	254	257
Mean	264	253
SD	11.983	12.420
% RSD	4.5	4.9
95% confidence interval (CI)	±12.58	±13.04
Overall mean	259	
Overall SD	12.937	
Overall RSD (%)	5.0	
Overall 95% CI	±8.22	

3.2.4. Precision (system precision, method precision and intermediate precision)

System precision was demonstrated by analyzing six replicate injections of monoethylsulfate standard solution (1000 ng/ml) as per procedure. The percentage relative standard deviation of six replicate injections of monoethylsulfate standard solution performed on six different days was found to be less than 2.0.

Repeatability of the test method (method precision) was demonstrated by analyzing six separate sample solution prepared using single batch of Indinavir sulfate. The percentage relative standard deviation of monoethylsulfate content in six sample preparations was found to be 4.5.

Intermediate precision of the test method was demonstrated by analyzing six separate sample solution prepared using single batch of Indinavir sulfate (that used for method precision), however by employing different analyst, different instrument, on different day with another lot of column. The percentage relative standard deviation of monoethylsulfate content in six sample preparations was found to be 4.9, while it was 5.0 for the cumulative of twelve preparations, and the results are summarized in Table 2.

Table 3
Recovery results from spiking of sample with monoethylsulfate.[a].

Spiked amount (μg/g)	Observed amount (μg/g)	Recovered amount (μg/g)	% Recovery
0	245		
251	497	252	100.4
502	730	485	96.6
607	818	573	94.4
Mean			97.1

[a] Three samples were prepared and analyzed at each spiking level. All values indicated are average of three data.

Table 4
The comparison of CE and IC method validation data.

Parameter	CE	IC
System precision (%RSD of injection)	<5	<2
Linearity range (μg/ml)	Between 4 and 15	Between 0.075 and 1.2
Recovery (%)	89.2	97.1
LOD (ng/ml)	880[a]	24
LOQ (ng/ml)	2422	74
Run time	8 min	25 min

LOD and LOQ values were calculated based on residual standard deviation method from linearity data from sensitivity experiment.

[a] Precision was not established.

3.2.5. Stability of sample solution

The stability of sample solution at room temperature (~25 °C) was evaluated by analyzing the sample solutions at different time intervals from initial (T0) to up to 22 h. The percentage difference between the results obtained from initial and different time intervals was found to be less than 10, suggesting that the sample solution is stable for at least up to 22 h at room temperature (~25 °C).

3.2.6. Accuracy

The accuracy of the method was verified by preparing sample solution spiked with known amount of monoethylsulfate at three different levels in the concentration range between 250 μg/g and 600 μg/g. Each concentration levels were prepared in triplicate and analyzed as per the method. The mean percent recovery was found to be 97.1, and the results are summarized in Table 3.

4. Comparison of IC and CE methods

An ion chromatography was found to be more sensitive for the determination of monoethylsulfate in Indinavir sulfate at very low levels. In addition, the performance of both the methods (i.e., by IC and CE) were compared, and are tabulated in Table 4, wherein the data demonstrate the advantages of IC method over CE method.

5. Conclusion

The level of monoethylsulfate in Indinavir sulfate is to be controlled/monitored during routine as well as during stability storage analysis to conform the desired purity of active moiety. This optimized ion chromatography is simple, uses less reagents, shorter acquisition time, very sensitive and accurate. The results obtained from validation experiments prove that the ion chromatographic method used to determine the content of monoethylsulfate in Indinavir sulfate is selective, sensitive, linear, precise and accurate. Hence, this optimized Ion chromatography method is suitable and reliable to determine the content of monoethylsulfate in Indinavir sulfate during routine as well as during stability storage analysis.

Acknowledgements

The authors express their sincere gratitude to Aurobindo Pharma Ltd. Research Centre located in Hyderabad for providing the analytical support to pursue this work and we are also grateful to colleagues who helped us in this work.

References

[1] D. Askin, K.K. Eng, K. Rossen, R.M. Purick, K.M. Wells, R.P. Volante, P.J. Reider, Highly diastereoselective reaction of a chiral, non-recemic amide enolate with (*S*)-glycidyl tosylate. Synthesis of the orally active HIV-1 protease inhibitor L-735,524, Tetrahedron Lett. 35 (1994) 673–676.
[2] C.M. Suter, E. Oberg, A quantitative study of the reaction between some primary aliphatic alcohols and sulfuric acid, J. Am. Chem. Soc. 56 (1934) 677–679.
[3] P.N. Evans, J.M. Albertson, The ethyl-sulfuric acid reaction, J. Am. Chem. Soc. 39 (1917) 456–461.
[4] D.J. Clark, G. Williams, Esterfication of sulphuric acid. Part II. Ethyl alcohol, J. Chem. Soc. (1957) 4218–4221.
[5] International conference on harmonization of technical requirements for registration of pharmaceuticals for human use, ICH harmonized tripartite guideline. Impurities in new drug substances Q3A (R2), step 4 2006.
[6] B. Strauss, S. Okubo, Protein synthesis and induction of mutations in *Escherichia coli* by alkylating agents, J. Bacteriol. 79 (1960) 464–473.
[7] D.K. Banerjee, M.J. Fuller, H.Y. Chen, Determination of diethyl sulfate ethyl hydrogen sulfate and sulfuric acid in mixtures, Anal. Chem. 36 (1964) 2016–2020.

[8] L. Chen, B.D. Johnson, N. Grinberg, G.R. Bicker, D.K. Ellision, Kinetic characterization of the esterfication of sulfuric acid by ethanol using capillary electrophoretic ion analysis, J. Liq. Chromatogr. Relat. Technol. 21 (1998) 1259–1272.
[9] D.J. Eatough, M.L. Lee, D.W. Later, B.E. Richter, N.L. Eatough, L.D. Hansen, Dimethyl sulfate in particulate matter from coal and oil-fired powder, Environ. Sci. Technol. 15 (1981) 1502–1506.
[10] T. Smith-Palmer, B.R. Wentzell, L.D. Hanson, C.D. Macpherson, The identification of alkyl sulfates and sulfonates in atmospheric samples by ion chromatography, Sci. Tot. Environ. 83 (1989) 185–190.
[11] International conference on harmonization of technical requirements for registration of pharmaceuticals for human use, ICH harmonized tripartite guideline. Validation of Analytical procedures Text and methodology Q2 (R1), step 4, 2005.