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Research Article

A NOVEL VALIDATED HEADSPACE GAS CHROMATOGRAPHY METHOD FOR THE SIMULTANEOUS QUANTIFICATION OF THREE ORGANIC VOLATILE IMPURITIES IN ENROFLOXACIN PURE AND PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

Objective: This article describes a novel, simple, and rapid gas chromatographic method for quantification of three organic volatile impurities (OVIs) present in enrofloxacin and its pharmaceutical dosage forms.

Methods: ZB-624 30 m×0.53 mm, 3.0 µ column used as stationary phase and flame ionized detector is used as detector at 250°C. The injector temperature is maintained at 180°C. The nitrogen gas was used as a carrier gas with a flow rate of 4.0 mL/min. The method involved a thermal gradient elution. The total run time is 21.14 min.

Results: The retention time of three OVIs taken individually and in spiked standard solutions were determined. The retention times are 2.30 min for methanol, 7.07 min for 1-butanol, and 8.48 min for toluene, respectively. The % relative standard deviation for six injections should be not more than 10%. The % recovery ranges from 85 to 115%. The correlation coefficient (r^2) for linearity is not <0.99. The limit of quantification was found to be 260 ppm for methanol, 101 ppm for 1-butanol, and 56 ppm for toluene. Other validation parameters is done like as precision, ruggedness, robustness, solution stability, and Tablet analysis.

Conclusion: All the obtained results are found within the acceptable limits. The proposed method has been successfully applied for the quantification of OVIs present in enrofloxacin pure and its pharmaceutical dosage forms.

Keywords: Enrofloxacin, Organic volatile impurities, Method development, Validation.

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INTRODUCTION

Enrofloxacin (Fig. 1) is chemically known as 1-Cyclopropyl-7-(4-ethyl-piperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid. Chemical formula is $C_{19}H_{22}FN_3O_3$ and molecular weight is 359.4 g/mol. Enrofloxacin is often used in veterinary medicine to treat several bacterial diseases, as abscess, renal failure, carapace injury, oral cavity inflammation, cerebral meninges inflammation, gastrointestinal tract inflammation, lungs inflammation, wounds, abrasions, skin, and mucous membrane infection [1]. Hence, many solvents are used in the synthesis of enrofloxacin drug substances and in excipients used in the production of drug formulations. Many of these solvents generally cannot be completely removed by standard manufacturing processes preferably at low levels. These solvents like as organic volatile impurities (OVIs) are encounter during manufacture and storage of active pharmaceutical ingredients. The OVIs in the active pharmaceutical ingredients or from other drug manufacturing processes can be harmful for the human health [2]. The first problem that was facing the simultaneous quantification of these OVIs analysis of enrofloxacin in quality control was the inability of the present official methods.

The OVIs specifications were set in accordance with the toxicity of solvents vary from a low ppm to thousands of ppm. In general, OVIs are divided into three classes. Those are class-1, class-2, and class-3. Hence, in the synthetic process of enrofloxacin, methanol (class-3), 1-butanol (class-3), and toluene (class-2) are used as OVIs. After the drying process, analysis needs to be performed to check if amounts of solvents used at any step of the production do not exceed acceptable limits. The static gas chromatography headspace (GC-HS) quantification of OVIs is nowadays mature technique well established in pharmaceutical analysis [3]. Hence, our aim is to simultaneous quantification these three OVIs in a single method using GC-HS with flame ionized detector. The specifications of the three OVIs are 3000 ppm for methanol, 1000 ppm for 1-butanol, and 500 ppm for toluene. The structures of three OVIs are shown in Fig. 2.

In the literature review, few methods are reported on enrofloxacin. Some are stability indicating methods and combination method with other drug is available. Chakravarthy *et al*. reported as stability-indicating reverse-phase high-performance liquid chromatography (HPLC) method for simultaneous estimation of enrofloxacin and its degradation products in tablet dosage forms [4]. Borges *et al*. reported as a simple and rapid HPLC method for the multi determination of enrofloxacin, ciprofloxacin, and oxytetracycline in raw materials and veterinary pharmaceutical formulations [5]. From these literature survey, there are no methods on quantification OVIs in enrofloxacin. Finally, we hope our method is novel and very sensitive technique. To the best of our knowledge, there are no reports on the validated simultaneous quantification of six OVIs in the enrofloxacin pure and pharmaceutical substances using GC-HS with flame ionized detector.

METHODS

Chemicals and reagents

Methanol, 1-butanol, toluene, and dimethyl sulfoxide were purchased from Sigma-Aldrich. Enrofloxacin pure drug was taken from a local well known research laboratory. Dimethyl sulfoxide is used as a diluent and blank.

Instrumentation and chromatographic conditions

Chromatography was performed on Shimadzu chromatographic system equipped with a Shimadzu GC-2010 system with FID, samples were

injected through a Teledyne Tekmar HT3™ HS. Data acquisition and integration were performed using GC solution software. The instrument parameters described below were set up to determine the OVIs.

Chromatographic conditions

Column: ZB-624 (30 m, 0.53 mm ID, 3 μm); carrier gas: Nitrogen; flow rate: 4.0 mL/min; injector temperature: 180ºC; split ratio: 1:5; oven program: Initial 60ºC hold for 6 min, increase the ramp rate 35°C/min up to 240°C, hold for 10 min; detector temperature: 250°C; air gas flow: 400 mL/min; hydrogen gas flow: 40 mL/min; total run time is 21.14 min.

HS sampler condition

Vial condition temperature: 95°C; needle temperature: 105°C; transfer line temperature: 110°C; vial conditioning time: 30 min; vial pressurize time: 3.0 min; inject time: 1.0 min; injection volume: 1.0 mL; GC cycle time: 45 min.

Preparation of standard solutions

Specifications for OVI'S

Methanol is 3000 ppm, 1-butanol is 1000 ppm, and toluene is 500 ppm.

Standard solution preparation

Weighed and transferred about each 750 mg of methanol, 250 mg of 1-butanol, and 125 mg of toluene into a 100 mL of the volumetric flask containing 70 mL of diluent and diluted to volume with diluent. Further taken 5.0 mL of the above solution into 50 mL of volumetric flask and diluted to volume with diluent.

The standard HS vials were prepared with 2 mL of the standard solution and seal the vial with aluminum closure. (The standard solution has been prepared with respect to enrofloxacin sample concentration).

Preparation of enrofloxacin sample solution (250 mg/mL)

Accurately weighed about 500 mg of enrofloxacin pure sample into a 10 mL headspace vial and add 2.0 mL of diluent and immediately sealed with aluminum closure.

Preparation of enrofloxacin tablet solution

Twenty tablets were weighed and powdered. An amount of powder equivalent to 500 mg enrofloxacin was accurately weighed and transferred to a HS vial, add 2 mL of diluent and immediately sealed with aluminum closure.

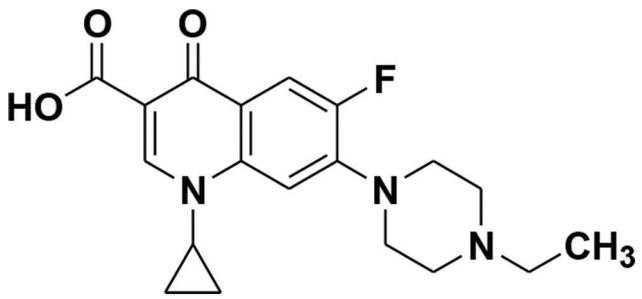

Fig. 1: Chemical structure of enrofloxacin

Calculation

The organic volatile impurity content was calculated from,

$$\text{PPM}(\text{OVI}) = \frac{\text{Impurity area in API}}{\text{Impurity area in standard solution}} \times \frac{\text{Standard solution concentration}}{\text{Sample solution concentration}} \times 10^6$$

GC-HS method development

This method development was implemented following quality-by-design principles including diluent selection and column selection. During the HS-GC method development, to select the most appropriate system parameters to obtain the best separation, sensitivity, and time efficiency, solvent mixtures were injected under a variety of conditions, for example, at different GC columns (DB-5, VF-1, ZB-624), HS temperatures, vial room temperature (70–90°C), needle temperature (80–110°C), transfer line temperature (90–130°C), detector temperatures (200–300°C), injector temperatures (100–230°C), GC gradients (40–230°C, at the rate of 10–40°C/min), carrier gas flow rates (2.0–4.0 ml/min), different diluents (N-Methyl-2-pyrrolidone, dimethyl sulfoxide, and dimethylformamide), etc. The final HS-GC conditions used for method validation were obtained based on optimized HS and GC parameters. Each of the solvents was injected once separately to determine method specificity and signal response sensitivity.

RESULTS AND DISCUSSION

Method validation

The method validation was done by evaluating specificity, repeatability, method precision, limit of detection (LOD) and limit of quantitation (LOQ), linearity, accuracy, ruggedness, and solution stability of residual solvents as indicated in the ICH harmonized tripartite guideline [2].

Specificity

Specificity of the method was shown by injecting the blank, sample preparation, and standard solution and showing the resolution between all peaks is in both sample solution and standard solution. The retention time of the three OVIs indicated that they were well separated from each other. The typical chromatograms of three OVIs and enrofloxacin are shown in Table 1 and Fig. 3.

System precision

System precision was determined by injecting six replicate injections of standard OVI solution, respectively, and analyzed as per ICH guidelines. The system precision of this method is expressed in the term of % relative standard deviation (RSD) of the data. The % RSD was found to should be <10 %. All values and chromatogram are shown in Table 2 and Fig. 4.

Method precision

Method precision has been demonstrated by separately analyzing of standard OVIs six preparations as per the method. %RSD was found to be <10%. All values and chromatograms are shown in Table 3 and Fig. 5.

Linearity for LOD and LOQ

Linearity of the method was determined over the concentration range of 5–25% for four OVI'S. Two replicates were performed at each

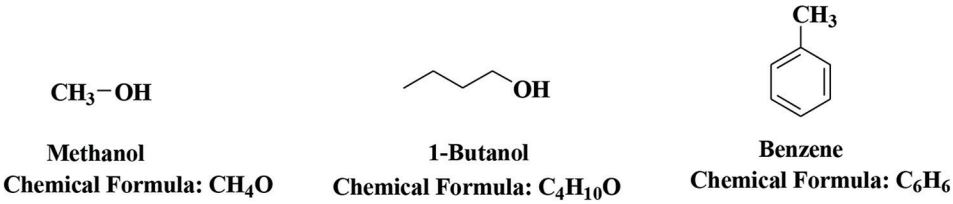

Fig. 2: Chemical structures of three organic volatile impurities

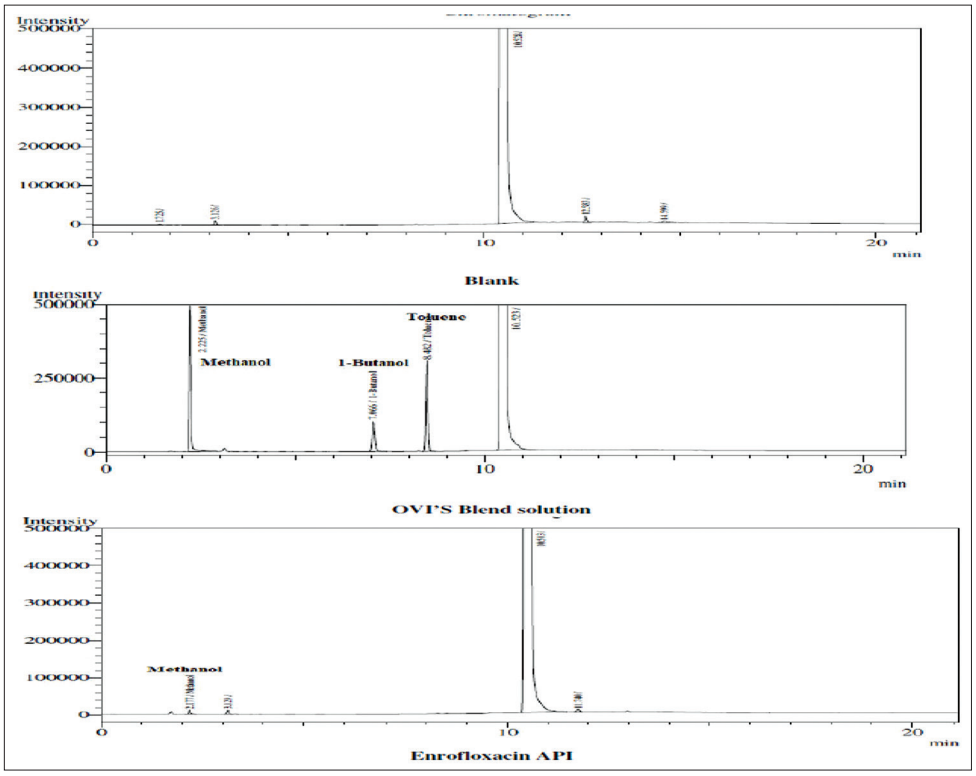

Fig. 3: Chromatograms for specificity

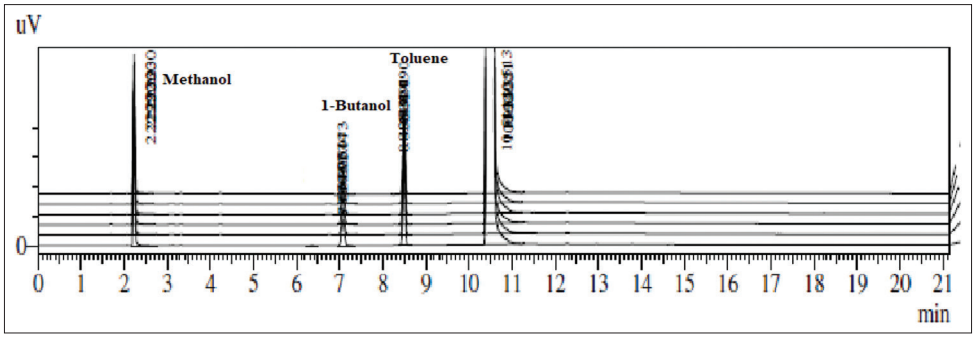

Fig. 4: Overlay chromatogram for system precision

Table 1: Specificity data for organic volatile impurities

S. No.	Name of organic volatile impurities	RT	Theoretical plates	Tailing factor	USP resolution
1.	Methanol	2.23	12976	1.25	---
2.	1-Butanol	7.07	49261	1.18	38.5
3.	Toluene	8.48	122966	1.05	11.25

Table 2: System precision data for organic volatile impurities

No. of injections	Methanol	1-Butanol	Toluene
1	1,378,901	436,880	1,077,497
2	1,197,929	399,464	996,593
3	1,330,807	424,521	1,084,919
4	1,343,481	440,384	1,077,712
5	1,368,134	432,735	1,099,049
6	1,256,913	412,634	1,061,559
ACVG	1,312,694	424,436	1,066,222
STDV	70,732	15,754	36,211
% relative standard deviation	5.39	3.71	3.40

level. Correlation coefficient (R^2), STEYX, SLOPE, LOD, and LOQ were calculated from these linearity data and shown in Table 4.

LOD and LOQ

The LOD and LOQ for the proposed method were determined using calibration standards and calculated using 3.3 σ/s and 10 σ/s formulae, respectively. The data and typical chromatograms are shown in Table 5 and Fig. 6.

Linearity with LOQ

The linearity of the method was determined by making injections of each organic volatile impurity over the range 25–150% and LOQ level. Two replicates were performed at each level. The calibration curves were obtained with the average of peak area ratios of two replicates. The correlation coefficient (r2) values for all OVIs were found to be higher than 0.99 and the calibration curves were linear within the range. All values and linearity graph are shown in Table 6 and Fig. 7.

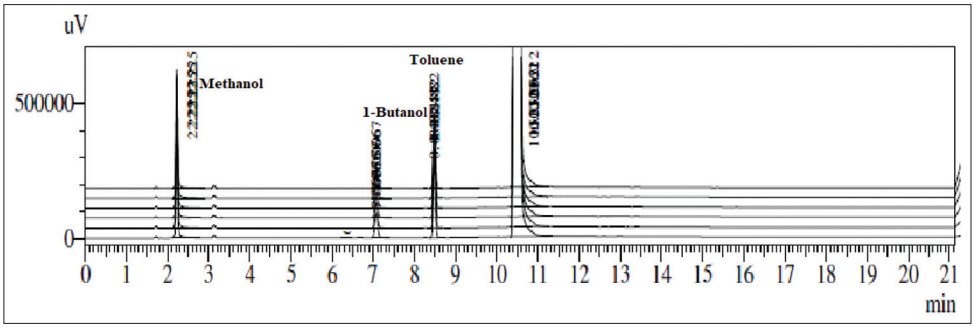

Fig. 5: Overlay chromatogram for method precision

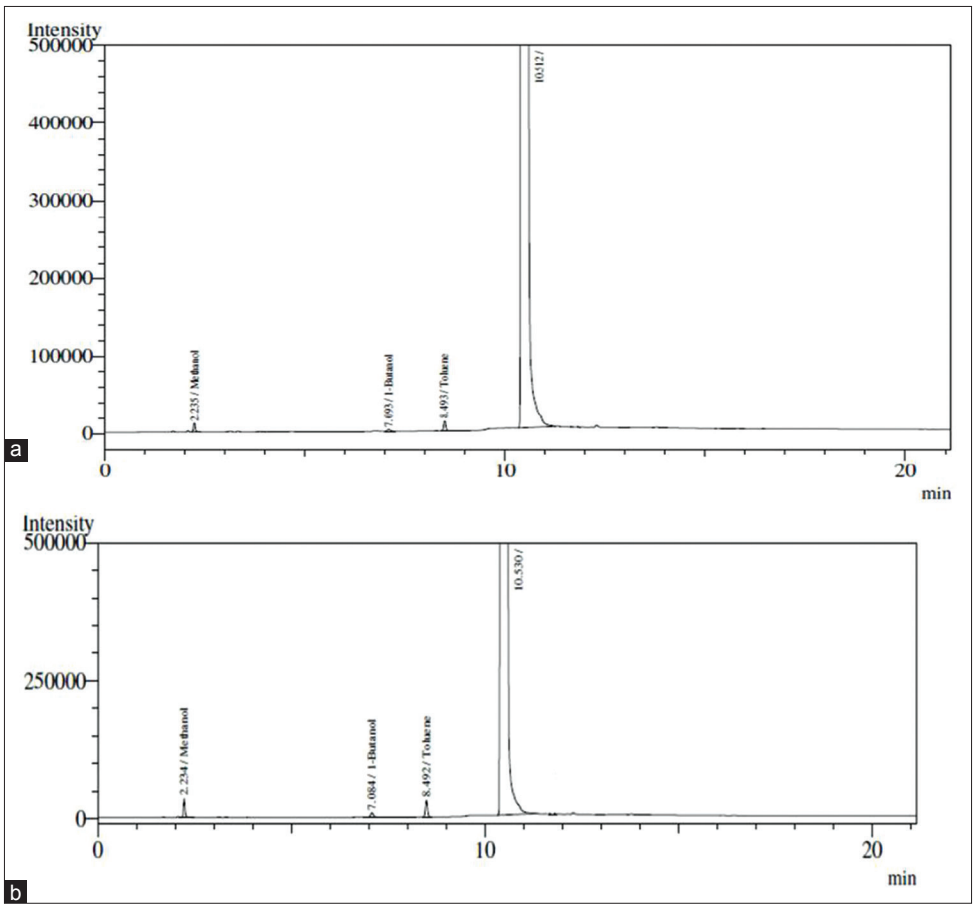

Fig. 6: (a) Limit of detection and (b) limit of quantitation chromatogram of three organic volatile impurities

Table 3: Method precision data for organic volatile impurities

No. of injections	Methanol	1-Butanol	Toluene
1	1,389,126	456,598	970,327
2	1,418,852	452,752	1,029,662
3	1,557,548	436,063	1,070,474
4	1,337,642	403,654	973,136
5	1,553,607	453,689	1,065,128
6	1,407,916	454,787	1,008,756
ACVG	1,444,115	442,924	1,019,581
STDV	90,735	20,637	43,512
% relative standard deviation	6.28	4.66	4.27

System precision at LOQ level

The system precision of this method is expressed in the term of % RSD of the data. System precision at LOQ concentration has been demonstrated by six replicate injections of standard solutions. The %RSD was found out to be <10 % of each impurity. Results and overlay chromatogram are summarized in Table 7 and Fig. 8.

Accuracy

Accuracy of the methods was assured by applying the standard addition technique. The enrofloxacin pure sample is spiked with three different levels (50%, 100%, and 150% and LOQ) of OVIs. The % recovery of each OVIs should be more than 85.0 and <115.0. Results obtained were within the limits indicating the method as accurate and are shown in Table 8.

Robustness

This study was performed by making small and deliberate variations in the method parameters. The variation in the column flow (±0.2 mL/min) and vial condition temperature (±5°C) was done and the results

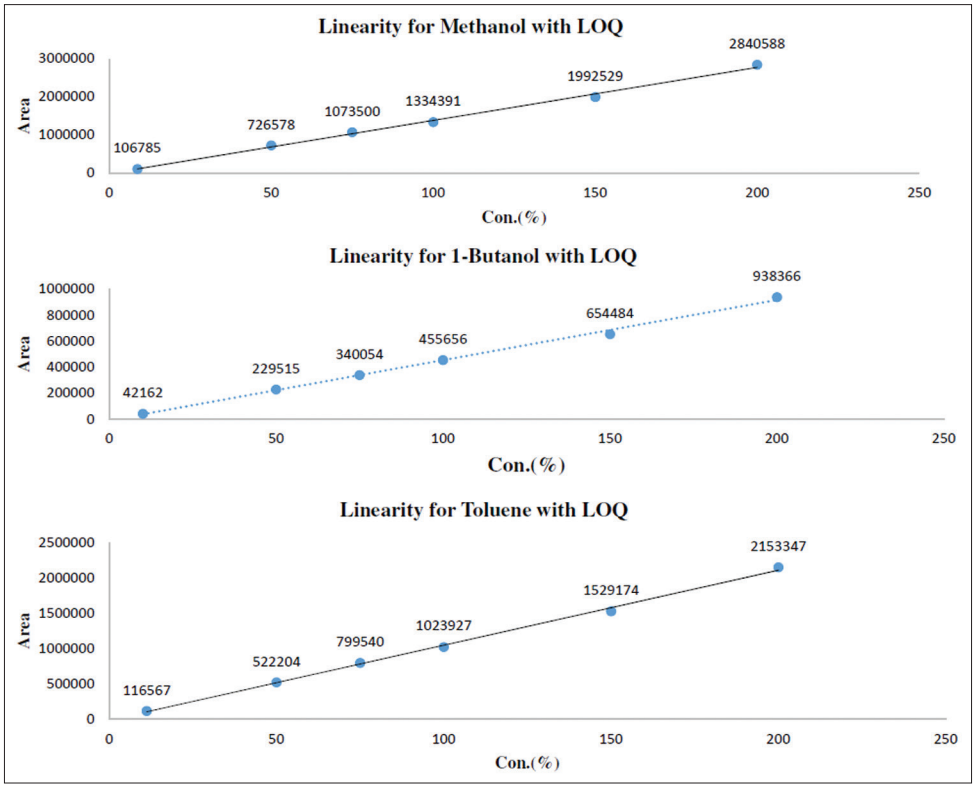

Fig. 7: Correlation graphs for three organic volatile impurities

Table 4: Linearity (low level) data for LOD and LOQ

Con. (%)	Methanol	1-Butanol	Toluene
	Avg. area (n=2)	Avg. area (n=2)	Avg. area (n=2)
5	84,011	21,676	74,485
10	136,735	37,410	97,244
15	232,120	63,366	157,109
20	290,334	83,428	211,139
25	348,871	95,814	254,035
r2	0.996	0.994	0.993
STEYX	11,867	3928	10,609
SLOPE	13,666	3886	9460
LOQ (%)	8.68	10.11	11.21
LOD (%)	2.87	3.34	3.70

LOD: Limit of detection, LOQ: Limit of quantitation

Table 5: LOD and LOQ data for three organic volatile impurities

OVIs	LOD Con. (ppm)	LOQ Con. (ppm)	LOD area	LOQ area
Methanol	86	260	34230	106785
1-Butanol	33	101	12460	42162
Toluene	19	56	48462	116567

LOD: Limit of detection, LOQ: Limit of quantitation, OVIs: Organic volatile impurities

Table 6: Linearity data for organic volatile impurities

Con.(%)	Methanol	1-Butanol	Toluene
	Avg. area (n=2)	Avg. area (n=2)	Avg. area (n=2)
LOQ Con.	106,785	42,162	116,567
50	726,578	229,515	522,204
75	1,073,500	340,054	799,540
100	1,334,391	455,656	1,023,927
125	1,992,529	654,484	1,529,174
150	2,840,588	938,366	2,153,347
r2	0.998	0.999	0.999

Table 7: System precision data at limit of quantitation

No. of injections	Methanol area	1-Butanol area	Toluene area
Run-1	103,352	38,842	113,023
Run-2	104,946	43,389	114,391
Run-3	106,537	44,340	116,826
Run-4	106,633	40,660	117,875
Run-5	107,479	42,307	117,107
Run-6	111,762	43,435	120,178
ACVG	106,785	42,162	116,567
STDV	2846	2058	2546
% relative standard deviation	2.67	4.88	2.18

were obtained within the acceptance criteria, indicating that the method is robust within the specified range. % RSD values were <10%, as shown in Table 9.

Ruggedness

Ruggedness of the method was evaluated by performing the sample analysis in six replicates using different analyst on different days and the results were obtained within the acceptance criteria, indicating that the method is rugged within the specified range. The % RSD is obtained not more than 10%. The results are presented in Table 10.

Enrofloxacin pharmaceutical application

The proposed method was evaluated by the assay of commercially available enrofloxacin tablet for quantification of OVIs present in it.

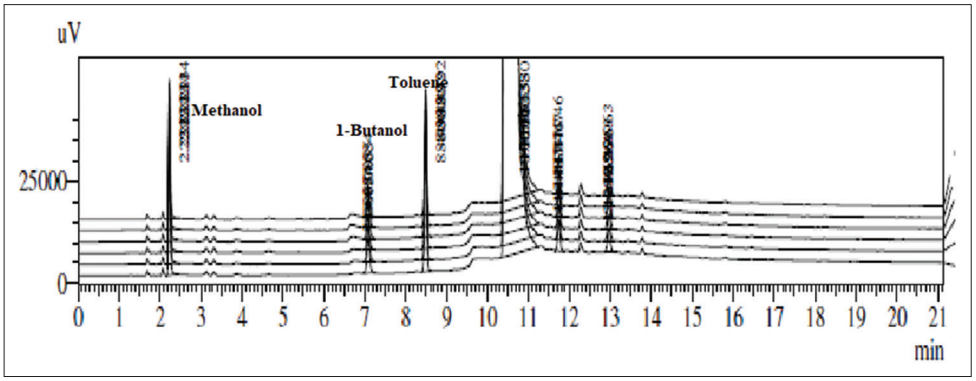

Fig. 8: Overlay chromatogram for limit of quantitation precision

Table 8: Accuracy data for OVIs

OVI'S	Avg. sample area (n=3)	Avg. STD area (n=6)	Avg. 50% area (n=3)	Avg. 100% area (n=3)	Avg. 150% area (n=3)	% recovery	
Methanol	28,597	1,312,694	695,859	1,421,799	2,027,423	50	101.66
						100	106.13
						150	101.51
1-Butanol	Not detected	424,436	199,414	448,800	639,257	50	93.97
						100	105.74
						150	100.41
Toluene	Not detected	1,066,222	480,364	1,000,517	1,485,189	50	90.11
						100	93.84
						150	92.86

OVIs: Organic volatile impurities

Table 9: Robustness data for three OVIs

Name of OVIs	Flow rate (mL/min)		Vial cond. temperature (°C)	
	3.8 mL/min (%RSD)	4.2 mL/min (%RSD)	90°C (%RSD)	100°C (%RSD)
Methanol	6.44	6.92	3.40	7.84
1-Butanol	3.10	3.22	5.25	5.32
Toluene	5.40	7.60	1.76	6.39

OVIs: Organic volatile impurities, RSD: Relative standard deviation

Table 10: Ruggedness data for four organic volatile impurities

Different days and analysts	%RSD for methanol	%RSD for 1-butanol	%RSD for toluene
Day-1			
Analyst-1 (n=6)	7.15	7.56	4.20
Analyst-2 (n=6)	2.93	3.23	1.64
Analyst-1 and2 (n=12)	5.35	5.61	3.07
Day-2			
Analyst-1 (n=6)	8.39	8.16	4.12
Analyst-2 (n=6)	4.59	5.26	4.01
Analyst-1 and 2 (n=12)	7.17	6.74	3.88
Analyst-1			
Day-1 and 2 (n=12)	7.83	7.73	4.09
Analyst-2			
Day-1 and 2 (n=12)	3.72	4.21	3.14

RSD: Relative standard deviation

The results obtained for OVIs were compared with the corresponding specification limits of standard guidelines and reported in Table 11. This revealed that OVIs present in enrofloxacin tablet at ppm levels which were less than the specified limits.

Table 11: Three organic volatile impurities content in tablet analysis

Name of drug	Label claim (mg)	Methanol (ppm)	1-Butanol (ppm)	Toluene (ppm)
Enrofloxacin (Enroquin™)	68 mg	601	Not detected	Not detected

CONCLUSION

Finally, from the above all method validation data, we have to concluded, this is the novel GC-HS method for the simultaneous quantification of six OVIs in enrofloxacin API. Methanol, 1-butanol, and toluene were well separated from each other and quantified by the proposed method. The good results are obtained in each validation parameter as per ICH guidelines. We reported that the LOD and LOQ value was very low level from this method. We have to prove this GC-HS method is also suitable for the quantification of OVIs in pharmaceutical dosage forms. The proposed method was validated as per the ICH guidelines and the results revealed that the method was scientifically. This investigation may be helpful to the manufacturers for controlling and minimization of the OVIs. Moreover, this method was found to be applicable for the routine analysis of the enrofloxacin API in the pharmaceutical industry.

AUTHORS' CONTRIBUTIONS

Dr. K. Prasada Rao supervised the manuscript preparation and reviewed manuscript. I would like to thank the whole staff of the Chemistry Department of Bapatla Engineering College for their technical support and productive discussions.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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