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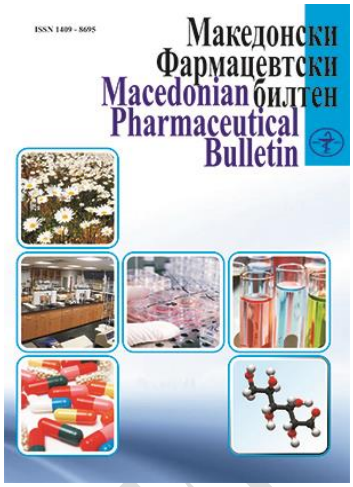

Title: Development and validation of a Headspace GC-FID method for the determination of acetone as a residual solvent in solid dosage forms in the presence of isopropyl alcohol

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Development and validation of a Headspace GC-FID method for the determination of acetone as a residual solvent in solid dosage forms in the presence of isopropyl alcohol

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Abstract

A simple and sensitive headspace gas chromatographic method was developed for the quantitative determination of acetone as a residual solvent, demonstrating selectivity in the presence of isopropyl alcohol (IPA). Acetone is used during one of the manufacturing steps of an immediate-release tablet, which represents the primary focus of this study, while IPA is specified as a residual solvent originating from the synthesis of the active pharmaceutical ingredient (API). A literature survey showed that, although several GC methods have been reported for the simultaneous determination of multiple residual solvents, only a limited number of methods are sufficiently sensitive and selective for the accurate quantification of a single target solvent.

Chromatographic separation was achieved using a DB-WAX capillary column (30 m × 0.32 mm, 0.5 µm film thickness). Helium was employed as the carrier gas at a flow rate of 2 mL/min with a split ratio of 30:1. The injector and detector temperatures were maintained at 180°C and 270°C, respectively. The oven temperature program consisted of an initial hold at 50 °C for 10 minutes, followed by a ramp to 210°C at 30°C/min and a final hold for 8 minutes. Headspace conditions included an incubation time of 15 minutes at 80°C, with loop and transfer line temperatures set at 90°C and 100°C, respectively. The injection volume was 1000 µL.

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The developed GC-HS-FID method demonstrates specificity, accuracy, precision, and linearity in accordance with ICH guidelines and is suitable for routine analysis of acetone in production samples of solid dosage forms.

Key words: gas chromatography-headspace (GC–HS), flame ionization detection (FID), residual solvents, acetone, isopropyl alcohol, solid dosage forms

Introduction

Organic solvents are widely used during the synthesis of active pharmaceutical ingredients (APIs), excipients, and throughout various stages of drug product manufacturing. Despite their functional importance, the presence of organic solvents in the final pharmaceutical product is undesirable due to their potential toxicity and associated risks to patient safety. Consequently, regulatory authorities require their strict control and limitation in drug substances and finished dosage forms. Various manufacturing and purification techniques are therefore employed to reduce solvent levels; however, trace amounts may remain even after extensive processing. These trace quantities are commonly referred to as residual solvents or organic volatile impurities and represent a critical quality attribute in pharmaceutical analysis and quality control. Accordingly, the reliable determination of residual solvents poses a significant analytical challenge in pharmaceutical development and routine quality assurance (Golembiovska et al., 2021; Grodowska and Parczewski, 2010).

Acetone is frequently employed as a granulation aid during the manufacturing of immediate-release solid dosage forms, which constitutes the primary focus of the present study. From an analytical perspective, gas chromatography (GC), particularly when coupled with headspace sampling, is the technique of choice for the determination of residual solvents due to its high sensitivity and suitability for volatile compounds. A review of the literature indicates that numerous GC-based methods have been developed for simultaneous screening of multiple residual solvents. The selectivity of such methods is generally focused toward the chromatographic separation of solvent mixtures commonly encountered in pharmaceutical manufacturing (Battu and Reddy, 2009; Jacobs et al., 2006; Lakshmi, 2025; Puranik et al., 2008). However, comparatively few studies

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address the development of methods specifically optimized for the accurate and sensitive quantification of a single residual solvent, particularly in the presence of structurally similar or co-existing solvents (Kieu To and Theruvathu, 2024; Valavala et al., 2018; Quirk et al., 2014).

In the present case, method development was further complicated by the presence of isopropyl alcohol (IPA), which is specified as a residual solvent originating from the synthesis of the API. Both acetone and IPA are classified as Class III solvents according to ICH Q3C guidelines, with acetone having a permitted daily exposure corresponding to a concentration limit of 5000 ppm (ICH Q3C (R6), 2018; ICH Q3C (R8), 2020). Although classified as low toxicological risk, accurate quantification of acetone remains essential for regulatory compliance and quality control. Literature data suggest that many existing GC methods do not provide sufficient chromatographic resolution between acetone and IPA, thereby compromising the unequivocal quantification of acetone in complex pharmaceutical matrices, especially in the presence of placebo interference.

The objective of this study was therefore to develop a simple, selective, and accurate gas chromatographic method for the determination of acetone as a residual solvent in an immediate-release tablet formulation in the presence of isopropyl alcohol. The developed method will subsequently be validated in accordance with International Council for Harmonisation (ICH) guidelines with respect to specificity, linearity, precision, accuracy, limit of detection (LOD), and limit of quantification (LOQ), thereby confirming its suitability for routine pharmaceutical quality control.

Materials and methods

Materials

The analyzed pharmaceutical dosage form was immediate release tablets containing 20 mg of BCS II class active ingredient. Acetone standard used for quantification was supplied by Sigma Aldrich. All reagents used were of analytical grade, supplied by Sigma Aldrich as well. The columns for GC analysis were supplied by Agilent. Gases used were of GC grade supplied by TehnoGas.

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Chromatographic method

Experiments were performed on Agilent 7890B equipped with a Flame Ionization detector (FID) and 7697 Headspace sampler (Agilent, United States). Instrument control, data acquisition and processing were done by using OpenLab Chemstation chromatography software (version A.01.09.126). Carrier gas was Helium at 2 mL/min with split 30:1. The separation was performed on Capillary column DB-WAX (30 m x 0.32 mm x 0.5 µ film thickness). Injector was set at 180°C and the detector temperature at 270°C. Temperature gradient was the following: Maintain the temperature at 50 °C for 10 minutes, then raise the temperature to 210°C at a rate of 30°C/min and maintain this temperature for 8 minutes. Incubation time was 15 minutes at a temperature of 80 °C, with the loop temperature at 90°C and transfer line temperature at 100°C. Injection volume was 1000 µL.

Preparation of solutions

Standard solution of acetone in final concentration of 4000 ppm was prepared for quantitative determination with Dimethyl formamide (DMF) as diluent. 1 mL of the standard solution was transferred in a headspace vial and sealed immediately.

Sample solutions were prepared by weighing the tablet directly into a headspace vial, then 1 mL of DMF was added and sealed immediately.

For checking the linearity of the method, a stock solution was prepared in concentration of 10 000 ppm and then dilutions were made to reach concentrations from 6000 ppm to 2.4 ppm (150 to 0.06% of working concentration). For checking the accuracy of the method, appropriate amount of placebo was weighed directly into a vial with addition of corresponding standard concentration.

Results and Discussion

Development

The presence of isopropyl alcohol as a residual solvent from API synthesis posed a significant analytical challenge due to its chromatographic proximity to acetone. Although acetone is associated with low toxicological risk, its reliable and unequivocal quantification remains critical to ensure regulatory compliance and robust pharmaceutical quality control.

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Identification and control of residual solvents - Ph Eur 2.4.24

The method development was initiated by using System A and sample preparation procedure 1 from Identification and control of residual solvents as described in Ph. Eur. 2.4.24.

Carrier gas was Helium at 35 cm/s with a split 5:1. A capillary column DB-640 (30 m x 0.32 mm x 1.8 µ film thickness) was used. Injector was set at 140°C and the detector temperature at 250°C. The temperature gradient was the following: Maintain the temperature at 40°C for 20 minutes, then raise the temperature to 240°C at a rate of 10°C/min and maintain this temperature for 20 minutes.

Incubation time was 60 minutes at a temperature of 80°C, with the loop temperature at 85°C and transfer line temperature at 90°C. Injection volume was 1000 µL.

There was a peak in the chromatogram of the sample solution as a rider peak to the peak of Acetone, confirmed to be originating from the matrix by injecting a placebo solution. (Fig. 1).

Fig. 1

Temperature gradient change

The initial temperature program was changed as follows: maintain the temperature at 40°C for 10 minutes, then raise the temperature to 240°C at a rate of 35°C/min and maintain this temperature for 5 minutes.

The peak form the matrix was no longer a rider peak to the peak of acetone and had satisfactory resolution. Standard solutions from 800 to 6000 ppm were injected to assess the linearity of the method. Peak splitting was observed in the chromatograms of standard solutions at concentrations higher than 1500 ppm (Fig. 2).

Fig. 2

Method optimization

To prevent the peak splitting, the column was changed to DB-640 (60 m x 0.53 mm x 3µm). Also, while the incubation temperature was kept at 80°C, the loop temperature was set to 90°C and transfer line temperature at 100°C to ensure total transfer of

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evaporated analyte. Incubation time was shortened to 20 minutes because it was shown that longer incubation time offered no benefits.

Different column flows from 0.5 mL/min to 2.5 mL/min were tried and the final flow was set to 0.5 mL/min as it offers best separation. The split ratio was increased from 1:20 to 1:50 to finally set at 1:70 to minimize the interference from the placebo.

The temperature gradient throughout the experiments was the following: Maintain the temperature at 40°C for 2 minutes, then raise the temperature to 210°C at a rate of 35°C/min and maintain this temperature for 5 minutes. Dimethyl sulfoxide (DMSO) was tried as a diluent and at first it offered better separation but the peaks of acetone had generally worse peak shape (Fig. 3).

Fig. 3

With this changes specificity was assured with the peak of acetone and the placebo peaks. Because in the specification of the active ingredient Isopropyl alcohol (IPA) was analyzed as residual solvent, it was also analyzed with our current method for specificity. IPA and acetone both had almost the same retention time (Fig. 4).

Fig. 4

Specificity with IPA

The temperature gradient was changed to introduce a rate of 5°C/min (maintain the temperature at 40°C for 2 minutes, then raise the temperature to 210°C at a rate of 20°C/min and maintain this temperature for 5 minutes), but the retention times of both analytes were still overlapping.

When isocratic method was applied with 40°C column oven temperature to gain separation, the peaks of acetone and IPA were separated to the baseline but with poor peak shape (Fig. 5).

Fig. 5

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Incubation temperature was lowered to 60°C and loop temperature to 80°C to ensure complete evaporation of acetone, but not of IPA. At 1 mL/min the peaks had satisfactory peak shape (Fig. 6).

Fig. 6

Even though the peaks were separated, the issue of the acetone peak splitting at higher concentrations arose again.

Change to polar column

The next step in development was aiming for application of a different column chemistry. DB-WAX (30 m x 0.32 mm x 0.5 µm) was used, as more polar and with thinner film layer.

Isocratic method was set at 60°C column temperature. Separation was achieved with 1.5 mL/min and no peak splitting was observed at high concentrations but the peak of placebo was again observed next to the peak of acetone (Fig. 7).

Fig. 7

Isocratic temperature method

The isocratic method was set to 50°C with injector temperature of 180°C, incubation temperature of 80°C and detector temperature of 270°C to narrow the peaks and separate the placebo from the acetone peak. With 2 mL/min the peak of the placebo was between the peak of acetone and the peak of IPA, with sufficient separation from both. (Fig. 8).

Fig. 8

To increase the sensitivity of the method it was tried to lower the split ratio. It was settled on a split of 1:30 that increased sensitivity without peak splitting at higher concentrations. To ensure adequate retention time of the diluent and to prevent column contamination a temperature gradient was introduced.

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The final conditions for the proposed chromatographic method are described in section 2. Materials and methods.

Validation

Specificity, Linearity, Accuracy, Precision and Limit of detection (LOD) / Limit of quantification (LOQ) were performed to ensure the reliability of the produced data.

Specificity

The parameter specificity is performed by preparation and analysis of diluent, sample and standard solutions, placebo solution and IPA as per test method. From the chromatograms it can be seen that no peaks were observed at the retention time of the acetone peak and the retention time of peak in the sample solution corresponds with retention time of peak from the standard solution (Fig. 9). Hence, it can be concluded that there is no interference with the peak of acetone.

Fig. 9

Linearity and range

Linearity was determined by series of injections of standards at concentrations that span from 0.2 - 150% of the expected working range. Peak area is shown as an average of 3 injections except at the limit of quantification (LOQ) where it is an average of six injections.

Linear correlation was obtained between the responses of acetone peak related to the concentrations of standards over the range of 8 ppm (LOQ) to 6000 ppm (Table 1). Correlation coefficient was 0.9999 opposed to the acceptance criteria of 0.99 (Table 1).

Table 1

Accuracy

Accuracy was evaluated by analysis of spiked samples in triplicate, by the proposed method, and the amount recovered was calculated at concentrations of 50, 100 and 150% of specification level. The recovery and the Relative standard deviation (RSD) results are

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within the acceptance criteria, so the test method has an acceptable level of accuracy (Table 2).

Table 2

Precision

The repeatability was shown by six replicate injections of the standard solution in concentration of 4000 ppm. For Method precision, six sample solutions were prepared individually using single lab trial batch of tablets, as per test method. RSD results of repeatability was 1.1% and of method precision was 4.1%, thus it can be concluded that the test method is precise (Table 3).

Table 3

Two sets of six sample solutions prepared individually using single industrial batch as per test method, were analyzed by different analysts, on two different batches of the column (Table 4).

Table 4

Limit of detection (LOD) / Limit of quantification (LOQ)

Quantitation and detection limits value was predicted from linearity data performed at lower concentrations. The LOD/LOQ were determined from the signal-to-noise ratio (S/N) method of 3:1 for LOD and 10:1 for LOQ. It was established that LOQ level is 8 ppm with S/N of 10.5 and LOD level is 2.4 ppm with S/N of 4.2 (Table 5). From the obtained data it can be concluded that acetone may be quantified to an acceptable degree of precision at this level.

Table 5

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Conclusion

The gas chromatographic method developed in this study successfully overcomes the limitations reported for existing approaches by providing adequate chromatographic resolution between acetone and isopropyl alcohol. The method is simple, selective, and accurate, and its validation in accordance with ICH requirements for specificity, linearity, precision, accuracy, limit of detection (LOD), and limit of quantification (LOQ) confirms its suitability for routine quality control of immediate-release tablet formulations.

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Резиме

Развој и валидација на Headspace GC-FID метод за одредување на ацетон како резидуален растворувач во цврсти дозирани форми во присуство на изопропил алкохол

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Клучни зборови: гасна хроматографија- headspace (GC-HS), детекција со пламен-јонизирачки детектор (FID), резидуални растворувачи, ацетон, изопропил алкохол, цврсти дозирани форми

Развиен е едноставен и чувствителен метод на гасна хроматографија со инјектор со статична глава (GC-HS-FID) за квантитативно определување на ацетон како резидуален растворувач, кој покажува селективност во присуство на изопропил алкохол (IPA). Ацетонот се користи во една од фазите на производство на таблети со комерцијално ослободување, што претставува главниот фокус на оваа студија, додека пак IPA е специфициран како резидуален растворувач кој потекнува од синтезата на активната фармацевтска супстанција (API). Прегледот на литературата покажа дека, иако се објавени повеќе GC методи за симултано определување на повеќе резидуални растворувачи, само ограничен број методи поседуваат доволна чувствителност и селективност за прецизна квантитација на еден целен растворувач.

Разделувањето беше постигнато со употреба на капиларна колона DB-WAX (30 m × 0,32 mm, дебелина на филмот 0,5 μm). Како носечки гас беше користен хелиум со проток од 2 mL/min и split однос 30:1. Температурите на инјекторот и детекторот беа одржувани на 180°C и 270°C, соодветно. Температурната програма

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на печката вклучува почетно задржување на 50°C во траење од 10 минути, проследено со покачување до 210°C со брзина од 30°C/min и финално задржување од 8 минути. Headspace условите вклучуваа време на инкубација од 15 минути на 80°C, при што температурите на loop-от и трансфер линијата беа поставени на 90°C и 100°C, соодветно. Волуменот на инјектирање изнесуваше 1000 µL.

Развиениот GC-HS-FID метод е специфичен, точен, прецизен и линеарен во согласност со ICH водичот и е соодветен за рутинска анализа на на ацетон во цврсти дозирани форми.

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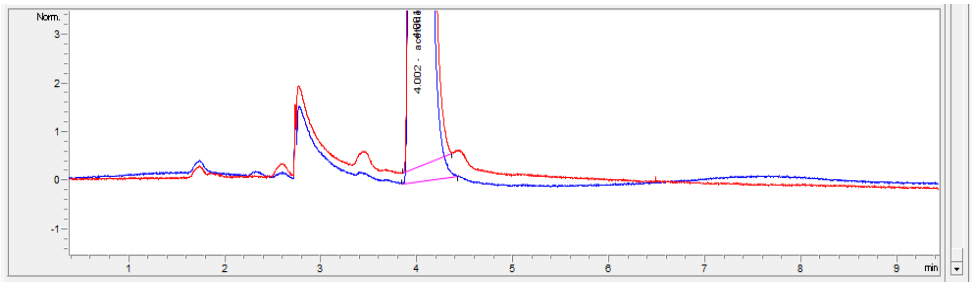

Fig. 1. Overlaid chromatograms of the Standard and Sample solution obtained using the method described in Ph. Eur. 2.4.24. Red: Sample solution, Blue: Standard solution.

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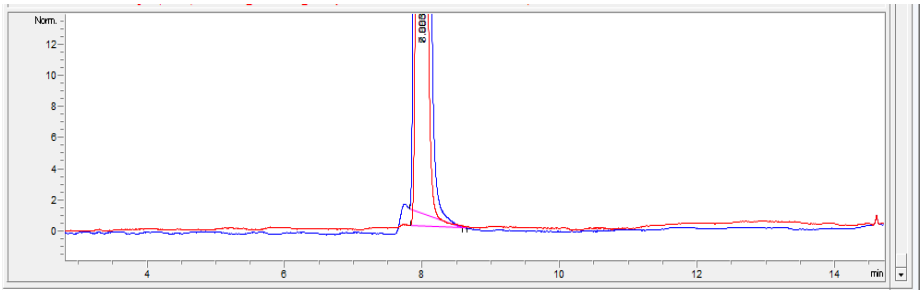

Fig. 2. Overlaid chromatograms of Standard solution, Red: 200 ppm Standard solution, Blue: 1500 ppm Standard solution.

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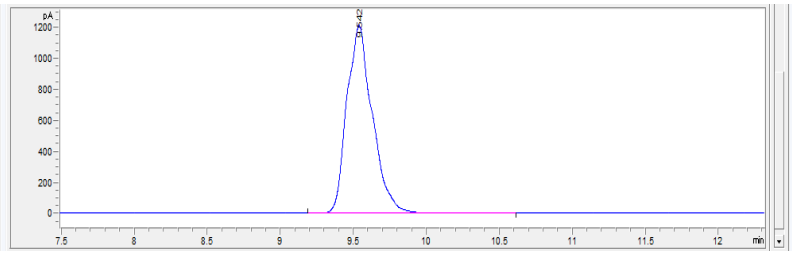

Fig. 3. Chromatogram of Standard solution with DMSO as diluent.

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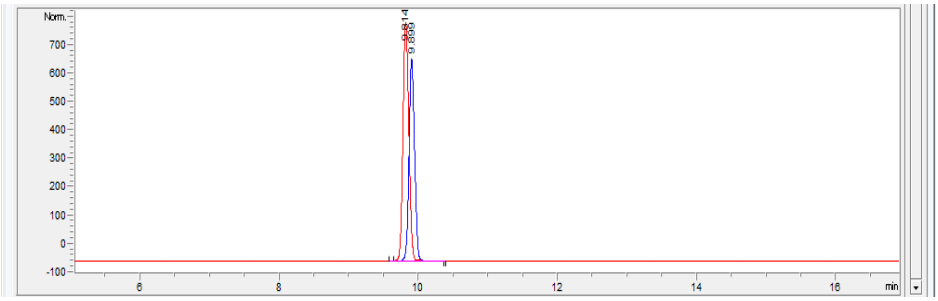

Fig. 4. Overlaid chromatograms of Standard solutions of Acetone and IPA, Red: Acetone peak (Rt about 9.8 min), Blue: IPA peak (Rt about 9.9 min).

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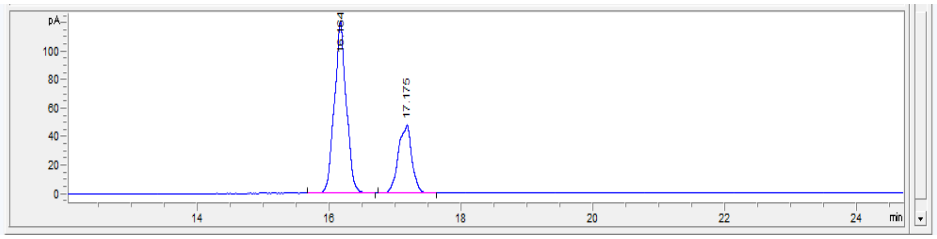

Fig. 5. Chromatogram of Standard solution containing Acetone (Rt about 16.2 min) and IPA (Rt about 17.2 min).

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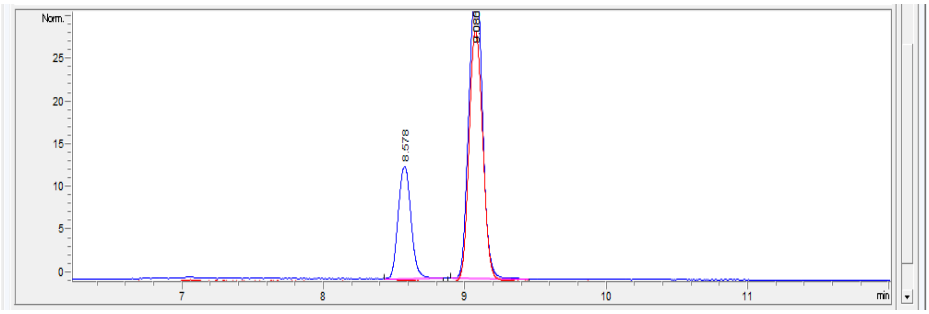

Fig. 6. Overlaid chromatograms of Standard solutions of Acetone (Rt about 8.8 min) and IPA (Rt about 9.1 min).

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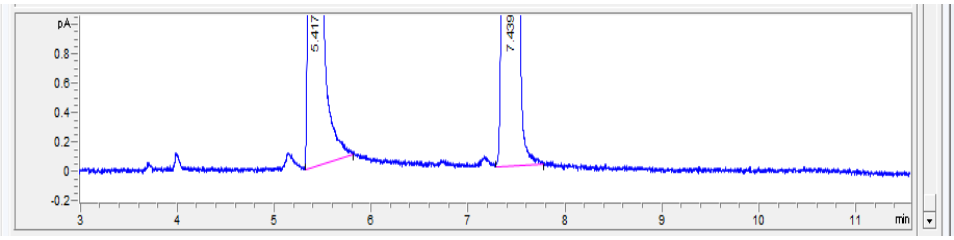

Fig. 7. Chromatogram of Placebo solution spiked with Standard solution containing Acetone (Rt about 5.4 min) and IPA (Rt about 7.4 min).

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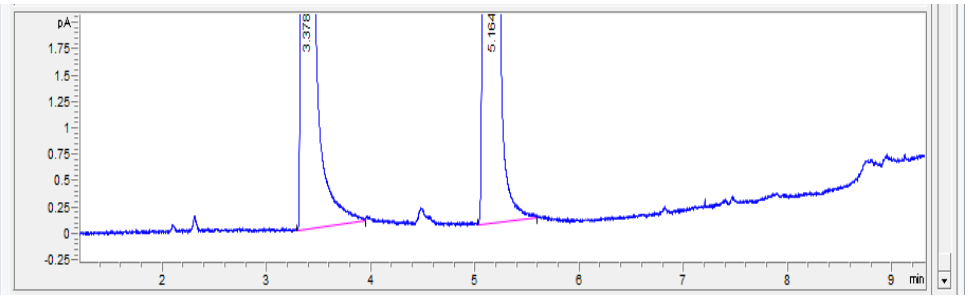

Fig. 8. Chromatogram of Placebo solution spiked with Standard solution containing Acetone (Rt about 3.4 min) and IPA (Rt 5.2 min).

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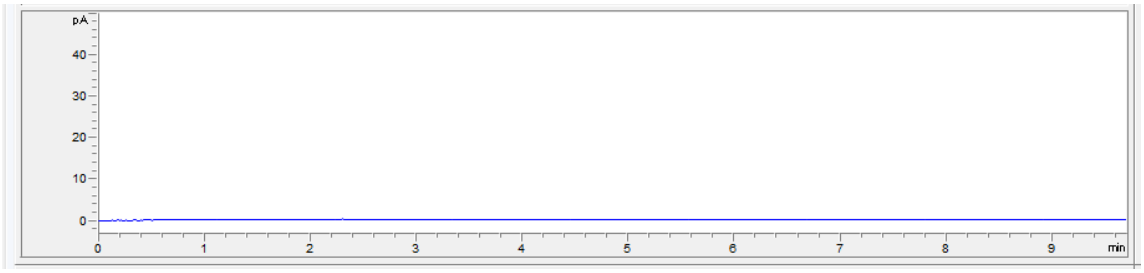
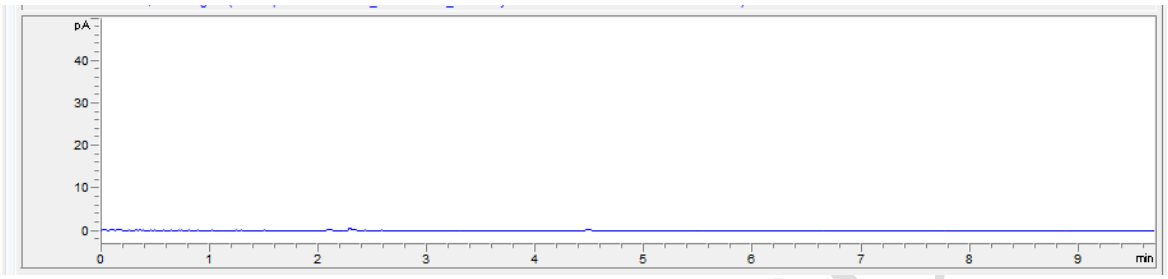


(a)



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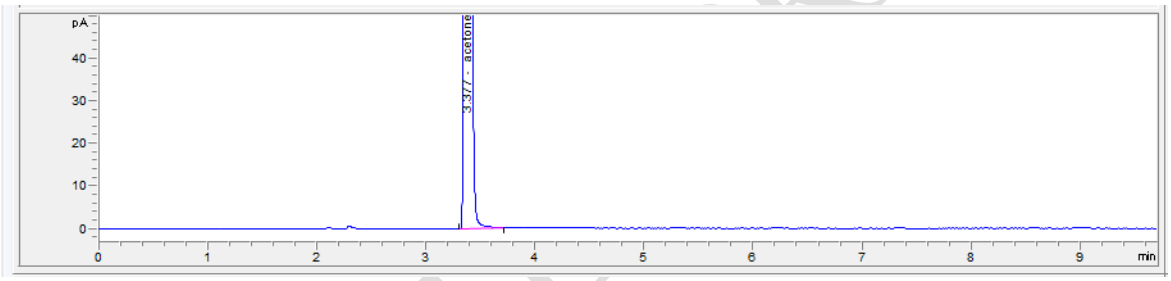

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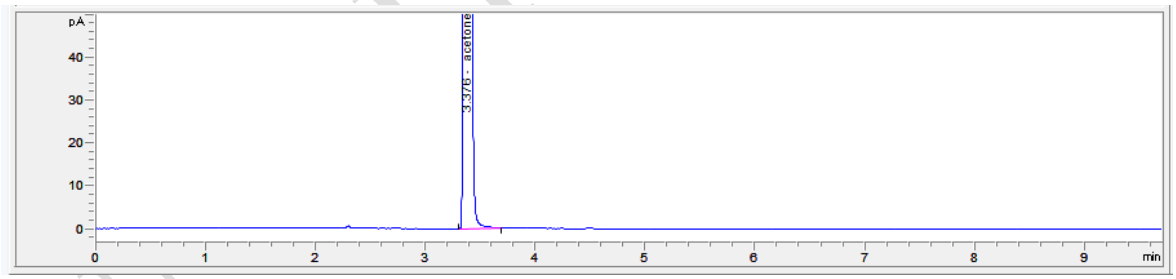

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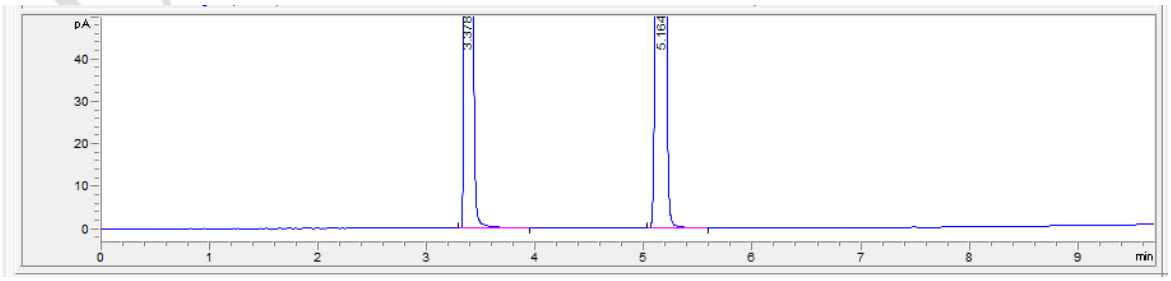

(e)

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Fig. 9. Validation of the method for determination of residual acetone - specificity demonstrated by the chromatograms obtained from: (a) diluent; (b) placebo solution; (c) standard solution (d) sample solution; (e) mix standard solution – Acetone peak (Rt about 3.4 min), IPA peak (Rt about 5.2min).

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Table 1. Linearity of the method for determination of residual acetone

Range (%)	Concentration (ppm)	Peak area
150	6000	3563
100	4000	2417
75	3000	1791
50	2000	1192
20	800	479
2	80	50
0.2	8	5
Statistical analysis		
Slope		0.5747
Intercept		3.7167
Correlation Coefficient (NLT 0.99)		0.9999

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Table 2. Accuracy of the method for determination of residual acetone

Recovery level (%)	Recovery (90.0-110.0%)	Recovery level (%)	Recovery (90.0-110.0%)	Recovery level (%)	Recovery (90.0-110.0%)
Spike 1 50%	105	Spike 1 150%	109	Spike 1 100%	104
Spike 2 50%	106	Spike 2 150%	105	Spike 2 100%	106
Spike 3 50%	105	Spike 3 150%	107	Spike 3 100%	104
Average	105	Average	107	Average	105
RSD (≤15.0%)	0.5	RSD (≤15.0%)	1.9	RSD (≤15.0%)	1

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Table 3. Precision of the method for determination of residual acetone - repeatability and method precision

Injection number	Area of standard solution	Amount in sample solution (ppm)
1	2292	4192
2	2335	4228
3	2335	4255
4	2343	3986
5	2367	3916
6	2356	3890
RSD (≤15.0%)	1.1	4.1

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Table 4. Validation of the method for determination of residual acetone - intermediate precision

Injection number	Analyst I, Column I (ppm)	Analyst II, Column II (ppm)
1	94	100
2	84	90
3	91	87
4	92	94
5	86	80
6	88	86
Average (%)	89	89
RSD (%)	4.4	7.1
Overall RSD (≤15.0%)	6.1	

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Table 5. Validation of the method for determination of residual acetone - LOD/LOQ

Range (%)	Concentration (ppm)	Area	Signal-to-Noise
LOQ 0.2%	8	4.672	10.5
LOD 0.06%	2.4	1.559	4.2

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