

A simple method for simultaneous determination of some organic liquids in in-process materials and effluents produced in the manufacture of amoxicillin and ampicillin

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Abstract A simple GC method for simultaneous determination of pivaloylchloride, methylacetoacetate, ethylacetoacetate and 2-ethylhexanoic acid in the presence of each other has been developed using glass column packed with 1% Silicone OV-17 on Gaschrom Q 100–120 mesh with temperature programming. Various performance parameters including precision, linearity and limit of detection have been evaluated. The method was found to be suitable for the analysis of these chemicals in in-process materials and effluents associated with the manufacture of amoxicillin and ampicillin. The run time was less than 15 min. The method has been successfully applied to determine the level of these hazardous organic liquids in real time samples.

Keywords Amoxicillin · Ampicillin · Effluents · Gas chromatography · Organic liquids

Introduction

Industrial effluents are considered among the major sources of pollutants of aquatic environment. A great deal of work is being carried out to identify and control the hazardous chemicals going into the natural waters. The industrial wastes may include organic and inorganic pollutants. Organic pollutants are of prime concern because of their high toxicity in trace quantities.

The present work aimed at identification and determination of the level of some of the most hazardous organic liquids in the effluent of a semisynthetic penicillin plant in Pakistan. This plant is engaged in the manufacture of ampicillin and penicillin for the last 15 years.

The manufacture of amoxicillin involves (Note) formation of (a) Dane's salt by the reaction of methylacetoacetate and D(-)-*p*-hydroxyphenylglycine in 2-propanol, (b) "mixed anhydride" by the reaction of Dane's salt with pivaloyl chloride in presence of pyridine, in dichloromethane and 2-ethylhexanoic acid, (c) amoxicillin ammonium salt by the reaction of "mixed anhydride" with 6-aminopenicillanic acid solution in dichloromethane, and (d) acid hydrolysis of amoxicillin ammonium salt to afford amoxicillin.

Similarly the manufacture of ampicillin involves formation of (a) Dane's salt by the reaction of D(-)- α -phenylglycine potassium salt with enolic form of ethylacetoacetate in 2-propanol, (b) "mixed anhydride"

The process details were provided by Dr. Tariq Mahmood the QA & Technical Manager of the plant under study.

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by the reaction of Dane's salt with pivaloylchloride (trimethyl acetyl chloride) in presence of pyridine, in dichloromethane and 2-ethylhexanoic acid, (c) ampicillin ammonium salt by the reaction of "mixed anhydride" with 6-aminopenicillanic acid solution in dichloromethane, and (d) acid hydrolysis of ampicillin ammonium salt to afford ampicillin.

From these processes pivaloylchloride, methylacetoacetate, ethylacetoacetate and 2-ethylhexanoic acid, the most hazardous organic liquids (Material Safety Data Sheet; Material Safety Data Sheet for Pivaloylchloride 1996 BASF; Material Safety Data Sheet for Ethylacetoacetate 1994; Material Safety Data Sheet for Methylacetoacetate 1996 Eastman; Material Safety Data Sheet for 2-Ethylhexanoic Acid 1997), end up in the finished product as impurities and in effluents as pollutants. It is desirable to have a good control on the presence of such impurities with a view to improve the quality of the product, prevent the loss of materials by an effective control of the process, and prevent the effluent water from contamination. It is also desirable to know the level of these contaminants at penultimate stage of semi-synthetic penicillins manufacture, precisely to control the processes.

To our knowledge there is no single method available in literature which allows simultaneous determination of these chemicals in presence of each other. The present study reports a rapid and precise gas chromatographic method for these compounds in the drug substances, in-process materials and effluents.

Materials and methods

Materials

The following chemicals were used without further purification.

Pivaloylchloride (BASF, Germany), methylacetoacetate (Lonza, Switzerland), ethylacetoacetate (Lonza, Switzerland) and 2-ethylhexanoic acid (BASF, Germany).

Standard solutions

Accurately measured volume, 10 mL each, of pivaloylchloride, methylacetoacetate, ethylacetoacetate and 2-ethylhexanoic acid, was made 100 mL in separate volumetric flasks with dichloromethane. Amoxicillin

and ampicillin, 50 mg each, were vigorously shaken separately for 15 min with dichloromethane (100 mL) and filtered through 0.2- μ m membrane filter. After the extraction the volume was made to 100 mL with dichloromethane.

Effluent solution

The effluent sample (50 mL) was mixed with dichloromethane (100 mL in three portions) vigorously shaken in a separating funnel and allowed to settle. The dichloromethane layer was separated and filtered through 0.2- μ m membrane filter. After filtration the volume was made to 100 mL with dichloromethane. This procedure was validated and recoveries were found to be: 75.36% (pivaloylchloride), 96.26% (methylacetoacetate), 95.84% (ethylacetoacetate) and 96.93% (2-ethylhexanoic acid). The lower recovery of pivaloylchloride is attributed to its partial decomposition into pivalic acid and hydrochloric acid in aqueous medium.

Chromatographic conditions

Column: Glass column 2 m \times 4 mm packed with 1% Silicone OV-17 on Gaschrom Q 100–120 mesh; *Detector:* Flame ionization; *Flow rate (N_2 , carrier):* 30 mL min⁻¹; *Injection volume:* 0.2 μ L; *Temperatures:* detector 150°C, injector 150°C, and column 60°C for first 4 min, raised to 200°C at 10°C min⁻¹ for 20 min.

The GC system used was Chrompack CP-9000 (Netherlands), equipped with flame ionization detector.

Procedure

Linearity

Ten different concentrations (10–100% v/v) of each substance in the mixture in dichloromethane were injected separately and the detector response was measured under standard conditions.

Limits of detection and quantitation

For determination of limit of detection (LOD) the standard solution of the analyte was diluted with dichloromethane to obtain a final response equal to twice the signal-to-noise ratio. Limit of quantitation (LOQ) was taken as 2 \times LOD.

Table 1 Performance parameters

Substance	Precision (RSD, %)				Resolution	Theoretical plates/column	LOD (L mL ⁻¹)	LOQ (L mL ⁻¹)
	Within day		Between days					
	Area	t _R	Area	t _R				
Pivaloylchloride (PC)	0.09	0.13	0.20	0.28	Between PC and MAA=8.26	679	0.20	0.40
Methylacetoacetate (MAA)	0.08	0.16	0.22	0.27	Between MAA and EAA=3.27	259	0.28	0.56
Ethylacetoacetate (EAA)	0.13	0.17	0.29	0.33	Between EAA and 2-EHA=6.55	776	0.31	0.62
2-Ethylhexanoic acid (2-EHA)	0.11	0.20	0.28	0.45	Between PC and 2-EHA=26.40	275	0.21	0.42

Precision

The standard solution (10%) was injected and the response was measured for ten replicates under the standard conditions.

Accuracy

Accuracy of the method for the determination of pivaloylchloride, methylacetoacetate, ethylacetoacetate and 2-ethylhexanoic acid was established in presence of each other by measuring the response of solutions of known concentrations of analytes in triplicate. The concentrations of the analytes were calculated and a linear regression was performed of the mean of these on analytes concentrations.

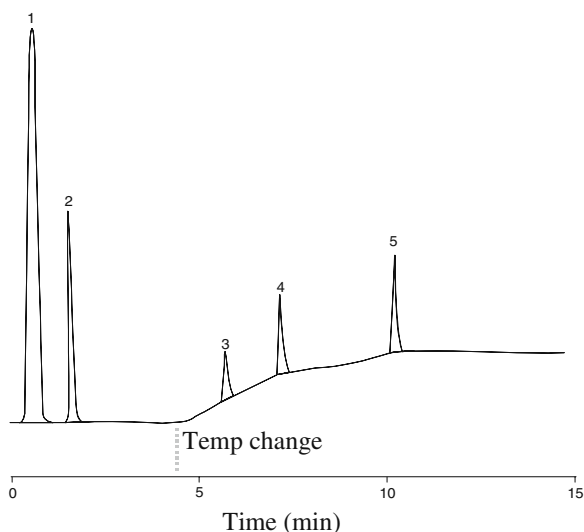


Fig. 1 Chromatogram of a mixture of peak 1 = dichloromethane, peak 2 = pivaloylchloride, peak 3 = methylacetoacetate, peak 4 = ethylacetoacetate, peak 5 = 2-ethylhexanoic acid, 10% each in dichloromethane

Results and discussion

All the substances under investigation were separated under the chromatographic conditions used. The resolution for a mixture of pivaloylchloride, methylacetoacetate, ethylacetoacetate and 2-ethylhexanoic acid ranged from 3.27 to 26.40 (Table 1). A representative chromatogram is shown in Fig. 1. The packed glass column was used because of being cheaper and readily available in all laboratories.

Linearity

From the linearity data a straight line was obtained. The statistical parameters including correlation coefficient, slope and intercept are given in Table 2. The response was found to be excellent as slope and correlation coefficient were very near to one and intercept was extremely lower than limit of $\pm 2\%$ of the detector response at 100% of the analyte level (Braggio et al. 1996).

Limits of detection and quantitation

The results are given in Table 1. The very low levels of LOD and LOQ indicate that the method is very sensitive for determination of substances under investigation and suitable for determination of these

Table 2 Linearity parameters in the 0–100% v/v concentration range

Substance	r ²	Slope	Intercept at zero concentration
Pivaloylchloride	0.9999	1.000	0.1693
Methylacetoacetate	0.9999	0.999	-0.0080
Ethylacetoacetate	0.9999	0.998	0.2040
2-Ethylhexanoic acid	0.9999	0.997	0.0506

analytes in effluents, in-process materials and finished products.

Precision

The within-day and between-days (10 days) precision of the method was determined for both peak area and retention time of all the analytes by measuring the response of replicate injections. The RSD values obtained were: 0.8–0.11% within day and 0.20–0.29% between days for peak areas; 0.13–0.20% within day and 0.27–0.45% between days for retention time (Table 1). The results are indicative of a very high precision of the method.

Accuracy

The resulting regression equations of the experimental data had slopes of 1 and intercepts of zero (within 95% confidence limits) with $r^2 \geq 0.997$ –0.999. This demonstrated a high accuracy of the method.

Analysis of real time samples

The real time batches of the drug substances and the effluents were analysed by this method. Of the 50 samples each of amoxicillin and ampicillin from consecutive batches analyzed none was found to contain the detectable quantities of pivaloylchloride, methylacetoacetate, ethylacetoacetate and 2-ethylhexanoic acid while pivaloylchloride (0.03–0.12%), methylacetoacetate (0.10–0.22%) and ethylacetoacetate (0.05–0.19%) were detectable in ten out of 50 samples of effluents. These organic liquids are highly toxic and there

presence in effluents may endanger the aquatic life. The degradation products of pivaloylchloride are hydrochloric acid, phosgene and carbon monoxide which are extremely dangerous for animal and human life (Material Safety Data Sheet for Pivaloylchloride).

Conclusions

A method for simultaneous determination of pivaloylchloride, methylacetoacetate, ethylacetoacetate and 2-ethylhexanoic acid in in-process materials and effluents produced in the manufacturing of amoxicillin and ampicillin has been developed and validated. The method is simple, rapid and precise. The linearity of the response and precision are good. The method can be applied for the routine monitoring of the analytes in process streams and effluents with good reliability.

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