

A rapid and valid HPLC method for the simultaneous determination of methanol, formaldehyde and formic acid in their mixtures without derivatization

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Abstract:

A new simple and reliable HPLC method is described and validated for the simultaneous determination of methanol, formaldehyde and formic acid in their mixture without derivatization. The method was validated in terms of its specificity, linearity, accuracy, precision, and limits of detection and quantification. The analysis cover concentration ranges: 0.00322 - 0.0516 M for formaldehyde, 0.0065-0.1051 M for formic acid, and 0.0067- 0.1085 M for methanol. The method uses a cation exchanger column (Eurokat)¹, with a 0.25 ml/min flow rate of 0.011 M sulfuric acid as a mobile phase in the presence of refractive index (RI) detector.

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Key words: Methanol, Formaldehyde, Formic Acid, HPLC, Refractive Index Detector, Derivatization, Simultaneous Determination

¹ Knauer Eurokat, H+ Form 300 x 800mm ID

طريقة HPLC سريعة وموثوقة لتحليل الميثانول والفورم الدهيد وحمض الفورميك في مزائجها ودون اشتقاق

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الملخص:

طورت طريقة تحليل بسيطة وموثوقة باستعمال HPLC من أجل تحليل كل من الميثانول والفورم الدهيد وحمض الفورميك ضمن مزائج تصنيعها وبشكل متزامن دون الحاجة لاشتقاق أي منها، تم التحقق من مصدوقية الطريقة التحليلية من خلال دراسة كل من نوعية وخطية وصحة ودقة الطريقة، وتعيين كل من حد الكشف الكمي والكيفي، يغطي التحليل مجال التركيز - 0.00322 - 0.0067 M 0.0516 M للفورم الدهيد، والمجال 0.1051 M-0.0065 M لحمض الفورميك والمجال - 0.0067 M 0.1085 M للميثانول، يُستعمل في الطريقة عمود مبادل كاتيوني (EUROKAT)² بمعدل تدفق 0.25 مل/د بوجود 0.011M من حمض الكبريت في الطور المتحرك، وباستعمال كاشف قرينة الإنكسار (RI).

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الكلمات المفتاحية: ميثانول، فورم الدهيد، حمض الفورميك، HPLC، كاشف قرينة انكسار، اشتقاق، تحليل متزامن

² Knauer Eurokat, H+ Form 300 x 800mm ID

Introduction:

Formaldehyde is an important material in both chemical and pharmaceutical industry. It is basically produced by the catalytic oxidation of methanol [1].

The product mixture of the oxidation process contains formaldehyde, formic acid, residual methanol and water. High performance liquid chromatography (HPLC) is the common choice for analysis of these individual compounds due to ease of conduct and lower cost. However, it necessitates derivatization of formaldehyde and methanol to increase the analytical sensitivity [2-7]. Although derivatization methods are sensitive and allow detection of trace amounts of these compounds, but they are not appropriate for daily analysis from the viewpoint of time and reagent consumption. It is therefore anticipated to avoid derivatization. Unfortunately, there is no appropriate HPLC method yet for simultaneous determination of formaldehyde, formic acid and methanol due to their different functionalities.

The aim of the present work is to develop a method for the simultaneous determination of formaldehyde, formic acid and methanol in product mixtures from catalytic oxidation of methanol using HPLC equipped with refractive index detector (HPLC-RI) without the need to employ derivatization. Refractive index detector is used to detect alcohols and aldehydes [8&9].

Materials and methods:

Methanol (99.999%), Formaldehyde solution (37–41%) and pure formic acid (98.0%) were obtained from (Panreac- Germany), sulfuric acid (Hi-media- India) and deionized water.

Instrumentation and chromatographic conditions:

Experiments were conducted using HPLC apparatus equipped with a cation-exchange column and RI detector, with the parameters given in Table (1).

Table (I): chromatographic parameters

Column	Knauer Eurokat, H ⁺ Form 300 x 800mm ID
Phase	Eurokat H, 10µm (poly phase)
Gradient	isocratic
Mobile phase	0.011 M sulfuric acid
Flow rate	0.25 ml/min
Stationery phase	Cation exchange column
Column temperature	10 °C
Injection volume	20 µL
detector	RI
Column pressure (bar)	52

Preparation of the standard solution:

The concentration of formaldehyde solution was determined by applying the iodometric method³ [11], the concentration was 35.7 % (wt. %).

A stock solution was prepared by diluting 5µL of each compound methanol, formaldehyde and formic acid in a 1000µL eppendrof⁴.

Working solutions were prepared by the dilution of the stock with mobile phase solution. Then a series of standard solutions for the three compounds were prepared at lower concentration by a serial dilution.

³ Formaldehyde is oxidized quantitatively to formic acid by excess of iodine in alkaline solution. The effective oxidizing agent is probably hypoiodite, and the formic acid formed is neutralized by the alkali present. When the oxidation is complete, the solution is acidified with sulfuric acid, and the liberated iodine is titrated with standard solution thiosulphate solution. This is the excess of iodine not utilized in the oxidation.

⁴ In this step only 5µL of each compound was taken, and the final volume was diluted to a 1000µL by deionized water in an eppendrof.

Method validation:

Specificity, linearity, sensitivity and precision were determined in the validation of the analytical method used for the simultaneous quantification of mixtures of formaldehyde, methanol and formic acid.

Specificity and separation:

Method specificity was achieved by a direct injection of definite mixture compositions of the standard solution. The HPLC chromatogram in Figure (1) shows no interference between the three peaks, and the compounds elute within 60 minutes. Formaldehyde elutes shortly before formic acid, and methanol elutes at the last. A small early peak at 17.2 min belongs to water present in original formaldehyde solution.

Resolution (R) is a quantitative measure of the degree of separation between two chromatographic peaks. Hence, it can be seen as a quantitative measure of separation's success. The resolution of the two peaks can be evaluated according to [11] using equation (1):

$$R = \frac{t_{r,2} - t_{r,1}}{0.5(w_2 + w_1)} \quad (1)$$

Where: $t_{r,2}$ and $t_{r,1}$ are the retention times of compound (2) and compound (1) respectively, and w_2 and w_1 are the peak width of them.

The calculated R for peaks (1) and (2) in HPLC chromatogram in Figure (1) amounted 1.47. Although formaldehyde partially overlaps with closely eluted formic acid, the developed method doesn't affect the separation resolution much more than around 0.13%, calculated according to the reference [11].

Thus, cation-exchange column shows a good performance in separation of aldehyde and alcohol which make it a good choice to analyze products of alcohol oxidation reactions. The obtained results allow a direct injection of the analyte with no need to derivative formaldehyde or methanol.

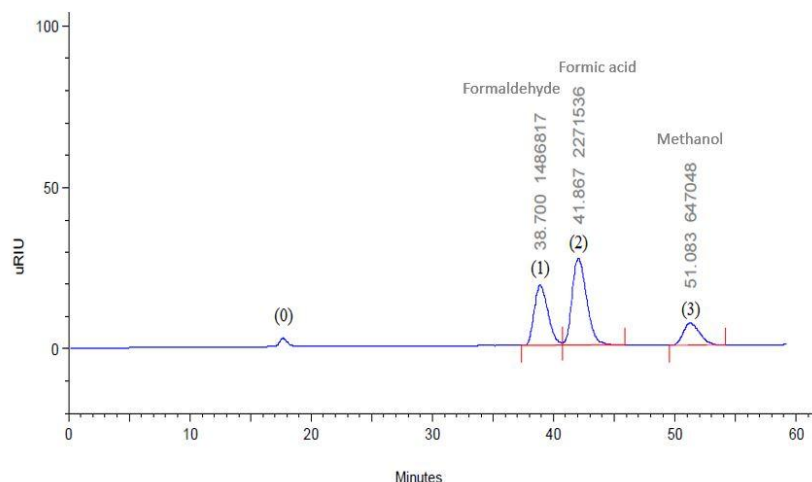


Figure (1): The chromatogram of a standard mixture of formaldehyde, formic acid, and methanol, with concentrations of 0.0516, 0.1051, and 0.1085 M, respectively.

Linearity:

Eight samples of each standard stock solution were directly injected in the column of the chromatograph. These samples cover a concentration range 0.00322 - 0.0516 M for formaldehyde, 0.0065-0.1051 M for formic acid, and 0.0067- 0.1085 M for methanol. The resulting correlation coefficients were $R^2=0.9982$, $R^2=0.9985$, $R^2=0.9979$ respectively.

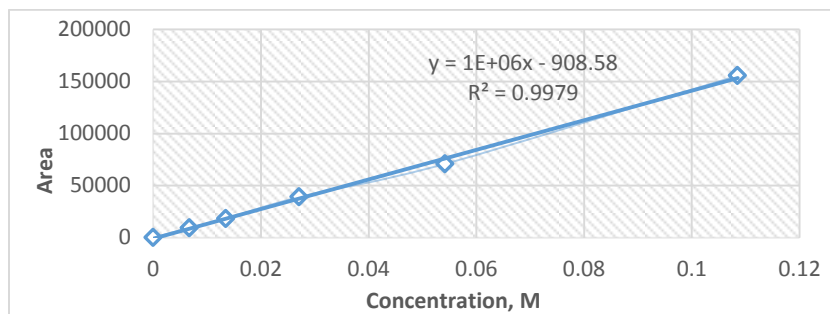


Figure (2): Methanol standard curve (0.0067-0.1085 M)

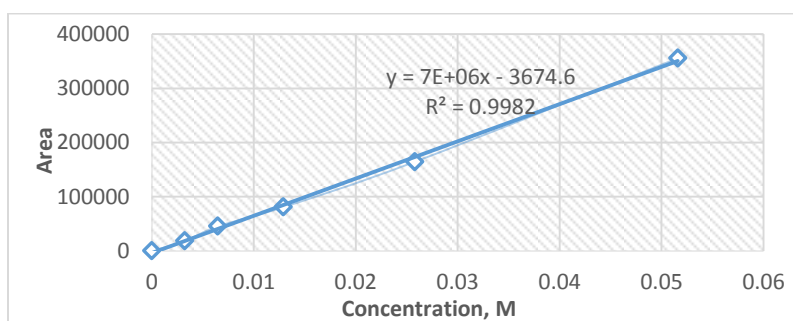


Figure (3): Formaldehyde standard curve (0.00322-0.0516 M)

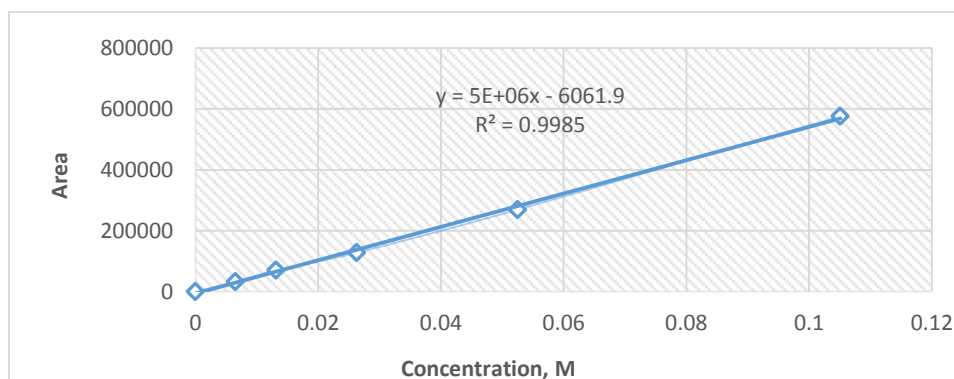


Figure (4): Formic acid standard curve (0.0065-0.1051 M)

Accuracy and precision:

The accuracy is measured as a percentage of the analyte sample recovered by volume, and expressed in terms recovery %, [12]. It was validated over the entire method concentration range.

The accuracy and precision study was evaluated by injecting a mix standard with known amount of concentration of methanol, formaldehyde and formic acid at four levels of concentration screening the whole calibration curves (n=3) accuracy was demonstrated by recovery percentage. All recovery values were within the acceptable range (96% to 104%), which means the method is accurate, and the precision of the procedure was achieved by repeatability, expressed by relative standard deviation (RSD%). Peak area showed (RSD%) $\leq 5\%$ and both are on the acceptable range.

Table (II): Method validation

	Real concentration (M)	Recovery (n=6)%	RSD ⁵ (n=6)%	LOD (M)	LOQ (M)	S/N ⁶ LOD	S/N LOQ
Methanol	0.0067	100.8	1.04	0.0020	0.0061	3.17	10.28
	0.107	103.2	0.61				
	0.0568	104.2	3.90				
Formaldehyde	0.0032	97.0	1.61	0.0010	0.0032	3.43	10.2
	0.0512	96.4	2.26				
	0.0272	96.0	1.15				
Formic acid	0.0065	97.4	0.65	0.0021	0.0063	3	10.93
	0.104	97.4	0.54				
	0.0552	98.0	0.28				

If the concentration of the sample was higher than the highest value in the calibration curve, sample can be diluted with HPLC water and easily detected.

Compared to the referenced method developed by Danyial hassan et al. our new method excels in terms of test quality and accuracy of analysis

Danyial hassan et al. had determined methanol and formic acid using HPLC with UV detector and C18 column with PDA detector. without derivatization of methanol, but it was shown that the method lacks sensitivity for high concentration of methanol, and formaldehyde interfere with the rest of the mixture [7].

trace amount of methanol also had been detected by Su-Hwei and his team, a sensitive method was develop based on derivatization of methanol using 3-bromomethyl-7-methoxy-1,4benzoxazin-2 under a strong alkaline treatment [3]. Applying this method for analyzing methanol oxidation reaction was difficult, because it takes long time to prepare derivatization agent, furthermore errors may happened during the analysis.

Conclusion:

In this study we could develop and validate a simple HPLC method to determine methanol, formaldehyde and formic acid, in one direct injection with no need to derivatize any compound, rather than applying a specific method or a specific equipment for each one. The method is adequate for analyzing methanol oxidation reaction products.

⁵ RSD: relative standard deviation

⁶ The average of three samples was taken

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