Pharmaceutica Analytica Acta
ISSN: 2153-2435
Amini, Pharm Anal Acta 2015, 6:11
DOI: 10.4172/2153-2435.1000442
Short Communication
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Identification of Ɛ-Caprolactam and Melamine in Polyvinyl- Pyrrolidone Powder by Double Injection Micellar Elektrokinetic Chromatography

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Abstract

A double-injection micellar *electrokinetic* chromatography (DIMEKC) method for the identification of Ɛ- *caprolactam* and melamine deliberately added to povidone (polyvinylpyrrolidone) products has been developed. The separations were performed in 89 mM phosphate buffer (pH 7.4) containing 52 mM sodium dodecyl sulfate (SDS) in fused silica capillaries with UV absorption detection at 200 nm. The identification relied on the agreement between the calculated migration time ($t_{mig(c)}$) of the analytes and the migration time (t_{mig}) of their corresponding reference standards being analysed simultaneously within a double injection run. The migration time of the analytes was calculated from the partial migration times ($t_{mig(p)}$) as described in this paper. The migration time ratios ($t_{mig(c)}$ / t_{mig}) varied between 0.997 and 1.005 (i.e., 1.001 ± 0.004), indicating good agreement between the observed and calculated migration times.

Keywords: DIMEKC; RMT; Povidone; Paracetamol; Ɛ-Caprolactam; Melamine

Introduction

Polyvinylpyrrolidone (povidone) as an important excipient is used in a variety of pharmaceutical formulations [1]. The European Pharmacopoeia recommends application of the Kjeldahl method [2] for the determination of povidone which contains approximately 12% nitrogen by mass [3]. The Kjeldahl method is because of its lack of specificity unable to selectively differentiate between different nitrogen containing compounds such as Ɛ-caprolactam, or melamine which increases the apparent amount of povidone [4]. Melamine and Ɛ-caprolactam contains 66% and 12% nitrogen by mass respectively [5]. These compounds are considered as possible economically motivated adulterants [2]. Melamine can cause kidney damage and renal failure [6]. Therefore, development of selective methods for the purity determination of povidone is of great importance to ensure its safety [2].

The aim of the present study has been to develop a CE method based on double-injection micellar electrokinetic chromatography for the identification of Ɛ-caprolactam and melamine as nitrogen containing compounds in povidone products [2,7].

Chemicals and Reagents

Paracetamol reference standard was obtained from the European Directorate for the Quality of Medicines and Health Care (EDQM, Strasbourg, France). Kollidon® SR, *polyvinylpyrrolidone* (average molecule weight 40,000), polyvinylpyrrolidone (average molecule weight 10,000), Ɛ-caprolactam and melamine were purchased from Sigma-Aldrich (St Louis, USA). Sodium hydroxide, disodium hydrogen phosphate and sodium di-hydrogen phosphate were purchased from Merck (Darmstadt, Germany). Sodium dodecyl sulfate solution (10% w/v) was purchased from BIO RAD (Hercules CA, USA). Water was purified by Elga Maxima LC water cleaning system. All samples to be analysed were filtered through a 0.22 µm MILLEX® GV filter (MILLIPORE, Carrigtwohill Co. Cork, Ireland). Before use, all sample solutions were stored in a refrigerator at 8°C.

Instrument

Capillary electrophoresis experiments were performed on a ProteomeLab PA 800 system (Beckman Instruments Inc., Fullerton, CA, USA), equipped with a photodiode array (PAD) system that monitored the wavelengths between 190 nm and 400 nm. Detection was performed at 200 nm. The background electrolyte (BGE) was prepared by adjusting the pH of 1.5 ml orthophosphoric acid (85%) to 7.4 with NaOH (3.0 M) and 37.5 ml of 100 mg/ml SDS solution. The volume was then adjusted to 250 ml with water. The final concentration of SDS was thus15 mg/ml (52 mM). A 118 cm fused silica capillary (108 cm effective length) x 50 µm I.D. (O.D. 375 µm) from Polymicro Technologies, Phoenix, AZ, USA was used. The capillary was cut to the desired length using a SGT Shortix capillary column cutter (Middelburg, The Netherlands).

Before the first injection, the capillary was consecutively preconditioned with water (5 min), and 0.5 M NaOH (10 min), followed by the BGE for 10 min at 80 psi (550.7 kPa). Injections were performed hydrodynamically at 1.2 psi (8.3 kPa) for 10 seconds. Immediately after the first injection a plug of the BGE was injected with pressure at 0.3 psi (2.1 kPa) for 3 seconds. Between the injections the capillary was washed with the BGE for 10 minutes. Separations were performed in normal polarity mode by applying +30 kV voltages across the capillary for 40 minutes. The capillary cartridge and the sample storage were thermostated at 22°C and 10°C, respectively. The separation vials were changed after every five runs.

In double injection mode, the separations are carried out by performing two injections. Between each injection the first injected sample plug was electrophoresed for 15 min (t_{PE}) [7] at 30 kV in order to keep the two sample plugs separated during the final analysis.

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Received October 28, 2015; **Accepted** November 23, 2015; **Published** November 26, 2015

Citation: Amini A (2015) Identification of Ɛ-Caprolactam and Melamine in Polyvinyl-Pyrrolidone Powder by Double Injection Micellar Elektrokinetic Chromatography. Pharm Anal Acta 6: 442. doi:10.4172/21532435.1000442

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Results and Discussion

Povidone, melamine and ε-caprolactam were separated at neutral pH (89 mM phosphate buffer) in the presence of 52 mM sodium dodecyl sulfate (SDS). The relatively high SDS concentration was necessary to baseline separate povidone and melamine which migrated close to each other. At the used pH value the EOF was sufficiently strong to sweep the negatively charged solute-SDS complexes towards the cathode. The counter current flow of the EOF and the SDS micelles contributed to the resolution of the solutes.

In order to enable the identification of the solutes the method was transferred to double injection mode by adjusting the time span for the partial electrophoresis (t_{PE}) of the first injected sample plug ($t_{PE} > \Delta t_{mig}$) [7,8]. The difference between the migration times (Δt_{mig}) was evaluated by taking into account the migration time of water and that of the last migrating solute, i.e., Ɛ-caprolactam (Figure 1). The first injected plug was therefore electrophoresed for 15 min ($t_{PE} > \Delta t_{mig} \approx 12$ min) prior to the second injection in order to avoid an inter-plug interference between the injected plugs. The full migration time ($t_{mig(c)Analyte}$) of the analytes was calculated from the partial migration time ($t_{mig(p)Analyte}$) by means the following equation:

$$tmig(c)Analyte = tmig(p)Analyte + t \quad (1)$$

the *t*-term was determined by using an either internal (IM) or external marker (EM) [7]. Paracetamol as a very weak acid eluted between melamine and Ɛ-caprolactam (Figure 1), therefore it was added as IM in both reference and analyte solutions. The magnitude of the t_{IM} is calculated by Equation (2). A more selective and analyte-related *t*-value may be obtained when reference standards are used as external markers [7].

$$tIM = tmig(IM) - tmig(p)IM \quad (2)$$

where $t_{mig(IM)}$ and $t_{mig(p)IM}$ stand for the observed and partial migration times of the IM, respectively.

$$tEMi = tmig(STD)i - tmig(p)STDi \quad (3)$$

where $t_{mig(STD)i}$ and $t_{mig(p)STD}$i are the observed and partial migration times of the reference standard, respectively.

The determined t_{EM} values were used for the calculation of migration times in the bracketing SDS-DIMEKC runs, i.e., in the runs before and after the reference run. The identification of the analyte was carried out by comparing the calculated migration times of the analytes and the observed migration times ($t_{mig(STD)i}$) of the reference standards:

$$RMT = tmig(c)Analyte / tmigSTD \quad (4)$$

The precision of the determined migration times and the RMT values are summarized in Table 1 and 2. As seen the RSD values for both calculated migration times and observed migration times of the solutes in the first and second injected plug, respectively, were less than 7% (Table 1). However, variations in the migration time differences (t_{EMi} or t_{IM}), being used for the migration time calculations, were less than 1%, i.e., significantly smaller than variation in the migration times (Tables 1 and 2).

The RMT ratios, determined using both t_{IM} ($RMT_{(IM)}$) and t_{EM} ($RMT_{(EM)}$), varied between 0.997-1.005 (Figure 2). The RMT ratios obtained using the external markers, as expected, were more accurate (1.0000-1.0001) than those determined applying t_{IM} (0.9990-1.0020) (Table 2). As Figure 2 demonstrates the $RMT_{(EM)}$ ratios, compared with $RMT_{(IM)}$ ratios, are more closely clustered around the average value. The closeness of the mean and median values indicates that the obtained RMT ratios are scattered more or less *symmetrically around* the *mean* values (Table 2 and Figure 2). This also indicates that variation in the data sets has mainly been caused by random errors.

Conclusion

The above experimental results demonstrated the ability of the double-injection SDS-MEKC method for the identification of Ɛ-caprolactam and melamine in povidone samples. As compared to the internal marker, the use of the external markers, because of their analyte specificity, provides more accurate results. The migration time ratios varied between 0.997 and 1.005 (i.e., 1.001 ± 0.004), indicating good agreement between the observed and calculated migration times (Figure 2). It is to be noted that the use of capillary electrophoresis in combination with other techniques sharply enhances trueness of the identification [9].

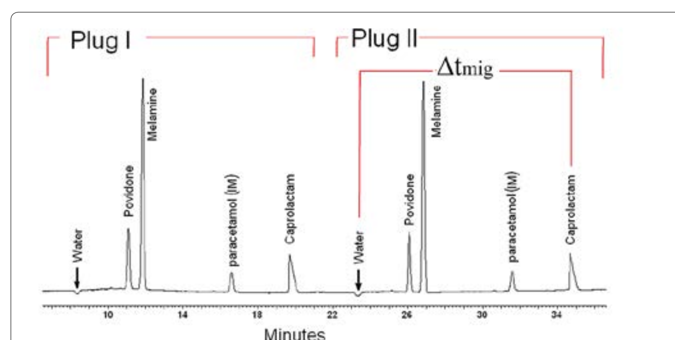

Figure 1: An example electropherogram from analysis of a mixture of povidone (20 mg/ml), melamine, paracetamol and ε-caprolactam in double injection mode. Running conditions: background electrolyte composed of 89 mM phosphate buffer (pH 7.4) containing 52 mM SDS. Other separation conditions as those given in the experimental section.

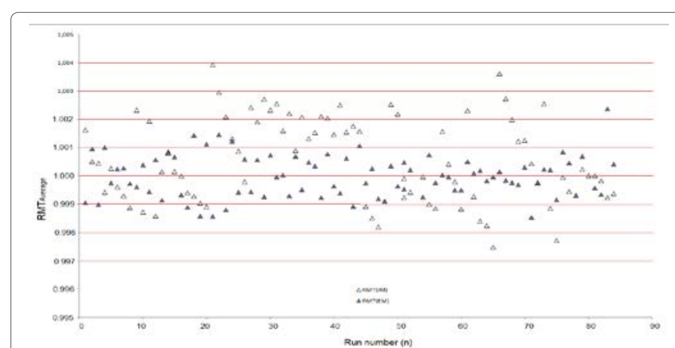

Figure 2: Run-to-run variation of the $RMT_{EM(average)}$ (▲) ratios and $RMT_{IM(average)}$ (**Δ**). $RMT_{(average)} = (RMT_{povidone} + RMT_{melamine} + RMT_{caprolactm})/3$. Operation conditions as those given in the experimental section.

Analyte	t_{mig} (± RSD%)	$t_{mig(c)}$ IM (± RSD%)	$t_{mig(c)}$ EM (± RSD%)
Povidon	24.111 (± 5.1%)	24.166 (± 5.1%)	24.096 (± 5.1%)
Melamin	24.830 (± 5.1%)	24.805 (± 5.2%)	24.809 (± 5.1%)
Paracetamol	28.746 (± 5.9%)	28.804 (± 5.8%)	28.722 (± 5.8%)
Caprolactam	31.322 (± 6.8%)	31.397 (± 6.6%)	31.239 (± 6.7%)

Table 1: Precision of observed and calculated migration times (n=80). The separation conditions as those given in Figure 1.

Analyte	RMT_{EM} ± RSD%	Median	RMT_{IM} (± RSD%)	Median	t_{IM} (± RSD%)
Povidon	1.0001 (± 0.10%)	1.0001	1.0006 (± 0.22%)	0.10001	14.95 (± 0.61%)
$t_{EM(Povidon)}$ (± RSD%)	14.94 (± 0.32%)	-	-	-	-
Melamin	1.0000 (± 0.10%)	1.0000	0.9999 (± 0.25%)	0.9993	-
$t_{EM(Melamin)}$ (± RSD%)	14.97 (± 0.82%)	-	-	-	-
Caprolactam	1.0002 (± 0.3%)	1.0001	1.0020 (± 0.15%)	1.0020	-
$t_{EM(Caprolactam)}$ (± RSD%)	14.86 (± 0.98%)				
Corrected t_{PE} (t_{PE} -0.5 t_{Ramp}) 14.92 min					

Table 2: SDS-DIMEKC identification of povidone, melamine and Ɛ-caprolactam. Paracetamol was used as internal marker (IM). The relative migration times were calculated by using either internal marker (RMT_{IM}) or external markers (RMT_{EM}). The separation conditions as those given in Figure 1, (n=80).

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