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Short communication

# Development and substantiation of a liquid chromatographic method for monitoring organic reactions involved in synthesis of 4-methoxyphenylacetic acid

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**Abstract**

A simple and rapid reversed-phase high-performance liquid chromatographic method for monitoring the reactions involved in two different processes for the production of 4-methoxyphenylacetic acid (PMPA) was developed. Impurity profiles of PMPA were used for fingerprinting of the two different synthetic processes by HPLC. Impurities were separated and determined on a Hypersil $C_{18}$ column with acetonitrile–0.1 *M* potassium dihydrogen orthophosphate–triethylamine (40:59.95:0.05, v/v) (pH 3.0) as the mobile phase and detection at 280 nm at ambient temperature. The method was substantiated with respect to accuracy, precision, linearity, robustness, limit of detection and quantification. The method was found to be suitable not only for monitoring the reactions but also for quality assurance of PMPA as it could detect impurities at the level of $4 \cdot 10^{-9}$ g.



*Keywords:* 4-Methoxyphenylacetic acid

## 1. Introduction

Phenylacetic acids belong to an important class of compounds that show a broad range of biological viz. antibacterial and analgesic activity [1,2]. These are used as important intermediates in the synthesis of anti-cancer calcium channel blockers [3]. The synthesis of these compounds generally involves (i) Rh catalyzed carbonylation [4], (ii) Pd/C catalyzed hydrogenation [5] and (iii) microwave induced Willgerodt–Kindler reaction under phase transfer catalytic conditions in a laboratory [6]. Recently, we have developed two different alternative procedures for the synthesis of 4-methoxyphenylacetic acid (PMPA) in our laboratory. During the development of these processes, an analytical method not only for monitoring the reactions but also for comparing the yields and purity of PMPA obtained by the two different processes was needed.

A thorough literature search has revealed that only a few analytical methods were reported for analysis of PMPA in environmental as well as certain physiological body fluids [7–15]. These methods include

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the use of liquid chromatography (LC), LC–mass spectrometry (MS), LC–nuclear magnetic resonance (NMR), gas chromatography (GC)–MS techniques for the identification and determination of PMPA in different matrices. In one of the earlier papers PMPA was determined in human plasma using pre-column derivatization with Nile blue followed by liquid chromatography [16]. PMPA was used as an extracting agent in the analysis of Al(III), Ga(III) and In(III) metals using gravimetry [17]. However, no method is available in the literature for the determination of its impurities and also monitoring of the reactions for process development. In the present paper, we describe an excellent reversed-phase high-performance liquid chromatography (RP-HPLC) method for the separation and determination of process related impurities of PMPA using a $C_{18}$ column with acetonitrile–0.1 *M* potassium dihydrogen orthophosphate–triethylamine (40:59.95:0.05, v/v) (pH 3.0) as the mobile phase and detection at 280 nm at ambient temperature. The accuracy and precision of the method was determined in accordance with ICH guidelines [18] and found to be suitable for quality assurance of PMPA.

## 2. Experimental

### *2.1. Materials and reagents*

All reagents were of analytical-reagent grade unless stated otherwise. Glass-distilled and deionized water (Nanopure, Barnsted, MA, USA), HPLC-grade acetonitrile, triethylamine (Ranbaxy, SAS Nagar, India) and potassium dihydrogen orthophosphate, orthophosphoric acid (S.D. Fine Chem, Mumbai, India) were used. Samples of PMPA, its reference standard (Aldrich, Milwaukee, WI, USA) and impurities viz. 4-methoxyacetophenone (PMAP), 4-methoxyphenylacetamide (PMAM), and 4-methoxyphenylthioacetomorpholide (PMTM) synthesized in our laboratory were used.

### *2.2. Apparatus*

The HPLC system was composed of two LC-10AT VP pumps, an SPD-10AVP diode array detector, an SIL-10AD VP autoinjector, a DGU-12A degasser and an SCL-10A VP system controller (all from Shimadzu, Kyota, Japan). A reversed-phase Hypersil $C_{18}$ (Thermo Quest, Runcorn, UK) column (25 cm×4.6 mm I.D.; particle size 5 μm) was used for separation. The chromatographic and the integrated data were recorded using a HP-Vectra (Hewlett-Packard, Waldbronn, Germany) computer system.

### *2.3. Chromatographic conditions*

The mobile phase was acetonitrile–0.1 *M* potassium dihydrogen orthophosphate–triethylamine (40:59.95:0.05, v/v), pH 3.0. Before delivering into the system it was filtered through a 0.45 μm PTFE filter and degassed using a vacuum. The analysis was carried out under isocratic conditions using a flow-rate of 1.0 ml/min at room temperature (28 °C). Chromatograms were recorded at 280 nm using an SPD-10A VP diode array detector.

### *2.4. Analytical procedures*

Solutions of PMPA (1 mg/ml) and impurities (0.5 mg/ml) were prepared in mobile phase. A 20-μl volume of each solution was injected and chromatographed under the above conditions. The system suitability was determined by using 0.1% PMAM spiked with the PMPA (1.0 mg/ml) and evaluated by making five replicate injections. The system was deemed suitable for use if the tailing factors for PMAM and PMPA are less than or equal to 1.2, the resolution was greater than 1.5 or higher and column plate numbers for main peak were more than 4000. Synthetic mixtures and process samples were analyzed under identical conditions. The quantities of impurities were calculated from their respective peak areas.

## 3. Results and discussion

### *3.1. Optimization of the chromatographic conditions*

Fig. 1 shows the chemical reactions followed in synthesis of PMPA in our laboratory. It could be seen from Fig. 1 that there are three compounds,

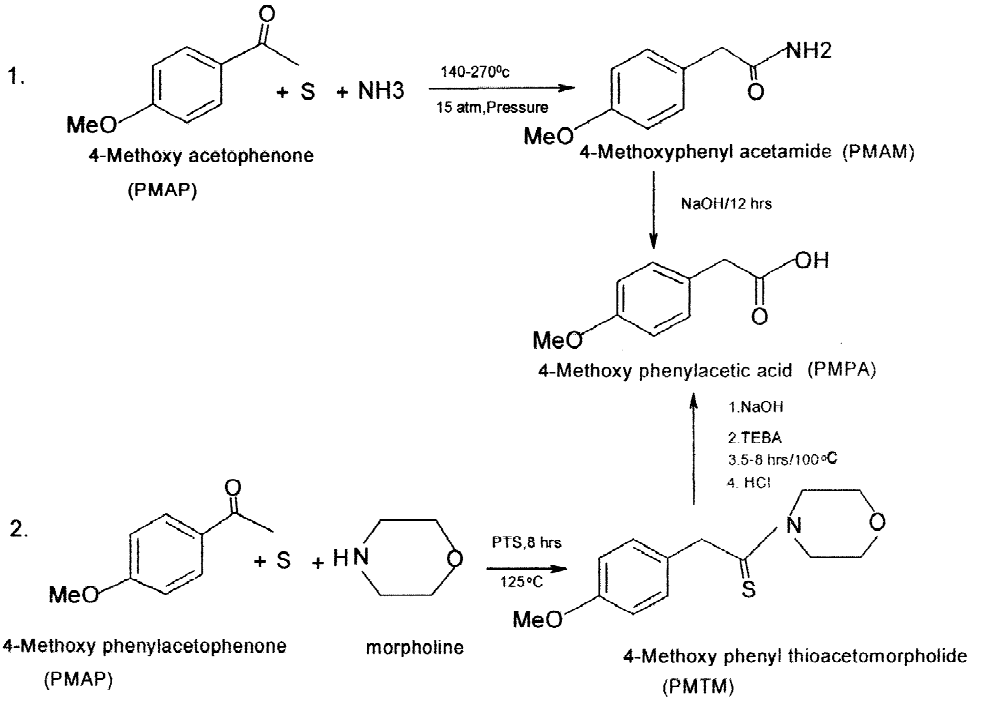


Fig. 1. Synthesis of PMPA through two different processes: (i) acetamide and (ii) thioacetomorpholide routes.

including the starting material and intermediates that could present as potential impurities in PMPA. The present study is aimed at developing a chromatographic system capable of eluting and resolving PMPA and its impurities originating from synthesis. In our preliminary experiments all the impurities and PMPA were subjected to separation by RP-HPLC on a Hypersil $C_{18}$ column with water–acetonitrile as eluent. Three compounds viz. PMAP, PMTM and PMPA were merged when the concentration of acetonitrile was kept below 40%. However, on increasing its concentration all three compounds were well separated, except PMAM. In another attempt, the water was replaced with 0.1 *M* potassium dihydrogen orthophosphate and the effect of concentration of organic modifier viz. acetonitrile on separation was studied (Fig. 2). When the concentration of acetonitrile was 40% all the impurities and PMPA were eluted and well separated. A typical chromatogram of a synthetic mixture containing PMPA and its impurities shown in Fig. 3. The peaks were identified by injecting and comparing with the retention times of the individual compounds. The

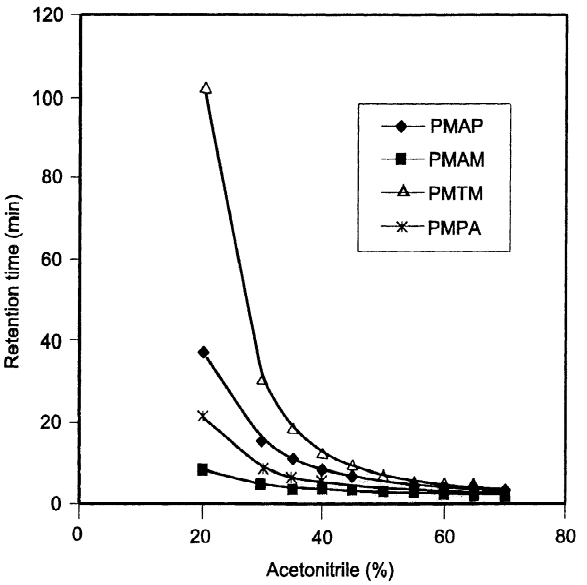


Fig. 2. Effect of concentration of acetonitrile on retention of PMPA, PMAM, PMAP, and PMTM.

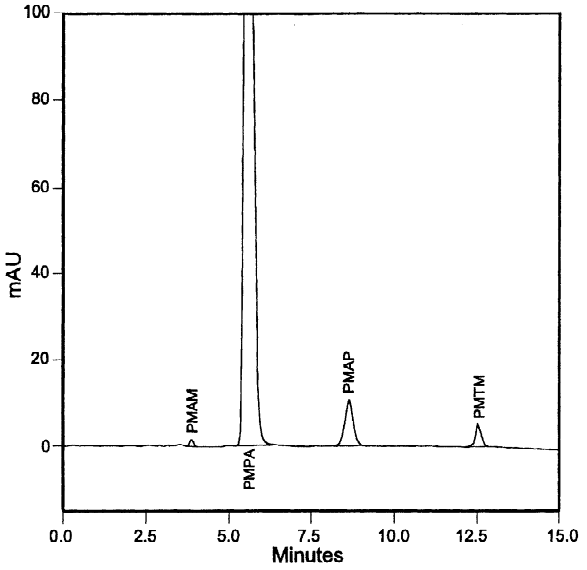


Fig. 3. Typical chromatrgroam showing 0.1% of: (i) PMAM, (ii) PMAP and (iii) PMTM spiked to PMPA.

order of elution was PMAM followed by PMPA, PMAP and PMTM. This was found to be in good correlation with their chemical structures. PMAM was eluted first, because of the highest polarity of the $-CONH_2$ group which gets protonated in the acidic (pH 3.0) mobile phase when compared to the others. Later, PMPA and PMAP containing –COOH and $-COCH_3$ were eluted according to the order of their polarities, respectively. PMTM being a non-polar molecule having the functional group of thiomorpholide eluted last at 12.63 min. The HPLC parameters under optimized conditions are (i) capacity factor 1.73 (PMPA), 0.89 (PMAM), 3.12 (PMAP), 5.06 (PMTM), and (ii) peak width 0.27 (PMPA), 0.18 (PMAM), 0.36 (PMAP), 0.48 (PMTM). The method was substantiated with respect to the system suitability, precision, accuracy, linearity, limits of detection and quantification.

### *3.2. Precision*

The system precision was determined by chromatographing 10 ($n=10$) injections of the standard PMPA solution and calculating the relative standard deviations (RSDs) of retention time, peak area, tailing factor, capacity factor, and the column plate numbers ($N$) for PMPA. The tailing factor was less than 1.2 and the plate number was more than 4000 for PMPA. Similarly the precision of the method was analyzed by 6 ($n=6$) injections of 0.1% solution of each impurity and the RSDs of retention time, peak area, tailing factor, and the resolution were calculated. The RSD ranged from 0.15 to 1.2%. The tailing factor was less than 1.2 and resolution was greater than 1.5 for all the components (Table 1).

### *3.3. Accuracy*

The recoveries of PMAP, PMAM, and PMTM were assessed by spiking the PMPA with each of the impurities at six different levels ranging from 0.025 to 0.15%. The recovery range and RSDs for all impurities were 86–105% and 0.29–5.85%, respectively (Table 2).

### *3.4. Linearity*

The linearity of peak area versus concentration was studied from 0.24 to 1.5 μg/ml for PMAP,

Table 1
Precision data

| | Compound | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | PMPA | | PMAP | | PMAM | | PMTM | |
| | Average* | RSD (%) | Average* | RSD (%) | Average* | RSD (%) | Average* | RSD (%) |
| $t_R$ (min) | 5.52 | 0.15 | 8.66 | 0.22 | 3.75 | 0.15 | 12.63 | 0.29 |
| RRT | 1.0 | 0.01 | 1.53 | 0.12 | 0.70 | 0.11 | 2.19 | 0.17 |
| Peak area | 793 563 | 0.71 | 114 531 | 1.46 | 7397 | 1.15 | 57 480 | 1.20 |
| Resolution | – | – | 2.75 | 0.45 | 1.53 | 0.34 | 6.15 | 0.43 |

*Average of six determinations.

$t_R$: Retention time, RRT: relative retention time.

Table 2
Recovery data

| | Nominal 0.1% of impurity spiked to PMPA | | | | | |
|---|---|---|---|---|---|---|
| | 25 | 50 | 75 | 100 | 125 | 150 |
| Amount added (μg/ml) | 0.25 | 0.5 | 0.75 | 1.0 | 1.25 | 1.50 |
| Recovery (%) (±RSD, %)* | | | | | | |
| PMAP | 98.65±1.30 | 97.62±2.08 | 97.62±0.29 | 103.36±1.15 | 101.02±0.62 | 101.73±0.98 |
| PMAM | 99.48±3.92 | 100.39±0.73 | 102.54±2.35 | 98.80±1.22 | 94.72±2.75 | 104.59±4.35 |
| PATM | 99.53±1.54 | 103.1±5.85 | 93.30±2.26 | 91.99±2.26 | 88.40±2.82 | 86.30±0.84 |

*$n=3$.

PMAM and PMTM. The data were subjected to statistical analysis using a linear-regression least-squares method. The calibration curves were found to be linear $y=27\,538x-27\,728$, $y=2022.9x-1402.4$ and $y=9459.8x-6187.9$ with correlation coefficients of 0.99, 0.97 and 0.99 for PMAP, PMAM and PMTM, respectively.

### 3.5. Limit of detection and quantification

The limit of detection (LOD) and limit of quantification (LOQ) were determined by measuring the magnitude of analytical background response (mean 0.044 mAU, RSD=6.8% ($n=4$)) by injecting blank samples. By substituting the mean value in the formula ($S/N=2\times$height of peak/$100\times$baseline noise) signal-to-noise ratio was calculated for each compound by injecting a series of solutions until the $S/N$ ratios 2–3 for LOD and 9.5–10.4 for LOQ were obtained. The LOD values determined were found to be 9.1 ng (PMPA), 4.5 ng (PMAM), 0.6 ng (PMAP), 2.0 ng (PMTM) and LOQs were 31.0 ng (PMPA), 15.4 ng (PMAM), 2.0 ng (PMAP), 7.0 ng (PMTM).

### 3.6. Stability

The stability of the PMPA and its impurities in the mobile phase was evaluated by analyzing solutions spiked with impurities at 0.1% of the specification level. The solutions were tested after 72 h at room temperature and the results demonstrated that the samples were stable at these conditions. Simultaneously the solution of PMPA in the mobile phase was stored for 24 h and chromatographed on the next day. No significant change was observed in the chromatogram.

The proposed HPLC method has been successfully adopted to determine potential impurities in different batches of PMPA. Fig. 4 shows the typical chromatograms of two samples obtained by two different processes. The total amount of impurities was found to be a maximum of 0.08% in one of the batches. From these results it could be seen that the developed method was simple and useful for monitoring the potential impurities of PMPA synthesized by two different processes.

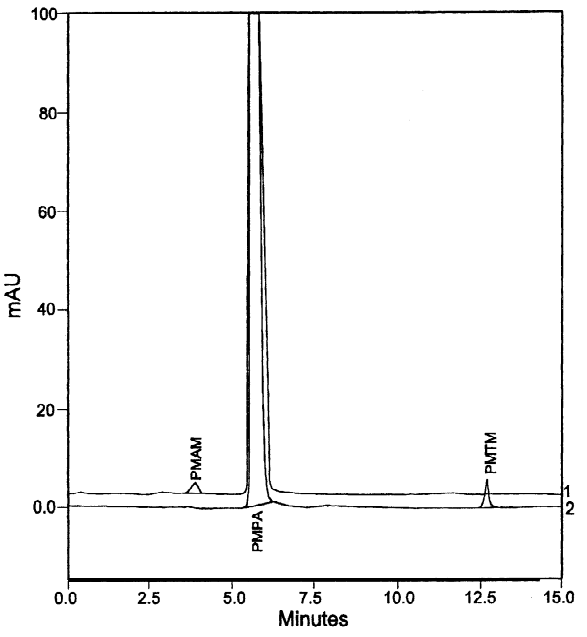


Fig. 4. Typical chromatograms showing of PMPA synthesized via: (1) acetamide and (2) thioacetomorpholide.

## 4. Conclusions

This work provides a comprehensive procedure for determining the impurities originated from the synthesis of PMPA by two different schemes. The method was found to be linear, accurate, reproducible, and capable of separating of impurities associated with the PMPA. Thus, the method can be used for process development as well as quality assurance of PMPA.

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