Pharmacogn. Mag.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
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ORIGINAL ARTICLE

Storing of Extracts in Polypropylene Microcentrifuge Tubes Yields Contaminant Peak During Ultra-flow Liquid Chromatographic Analysis

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Submitted: 12-01-2016 Revised: 10-02-2016 Published: 07-07-2016

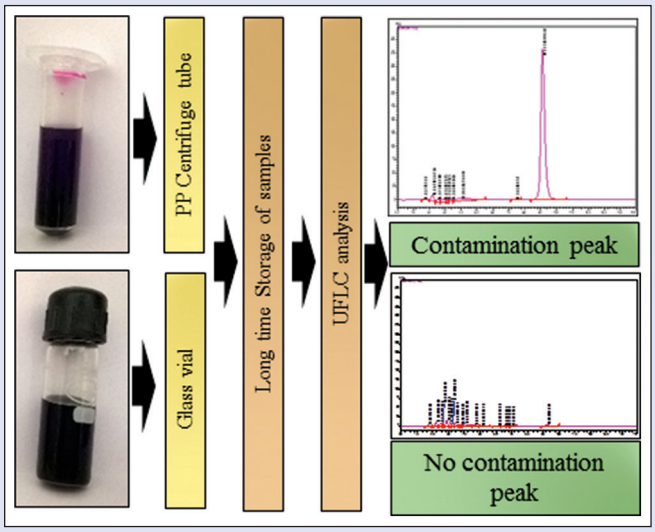
ABSTRACT

Background and Aim: This study was designed to understand the effect of storage in polypropylene microcentrifuge tubes and glass vials during ultra-flow liquid chromatographic (UFLC) analysis. **Materials and Methods:** One ml of methanol was placed in polypropylene microcentrifuge tubes (PP material, Autoclavable) and glass vials (Borosilicate) separately for 1, 2, 4, 8, 10, 20, 40, and 80 days intervals stored at –4°C. **Results:** Contaminant peak was detected in methanol stored in polypropylene microcentrifuge tubes using UFLC analysis. The contaminant peak detected was prominent, sharp detectable at 9.176 ± 0.138 min on a Waters 250–4.6 mm, 4 µ, Nova-Pak C18 column with mobile phase consisting of methanol:water (70:30). **Conclusion:** It was evident from the study that long-term storage of biological samples prepared using methanol in polypropylene microcentrifuge tubes produce contaminant peak. Further, this may mislead in future reporting an unnatural compound by researchers.

Key words: Contaminant, polypropylene, ultra-flow liquid chromatographic

SUMMARY

- Long-term storage of biological samples prepared using methanol in polypropylene microcentrifuge tubes produce contaminant peak
- Contamination peak with higher area under the curve (609993) was obtained in ultra-flow liquid chromatographic run for methanol stored in PP microcentrifuge tubes
- Contamination peak was detected at retention time 9.113 min with a lambda max of 220.38 nm and 300 mAU intensity on the given chromatographic conditions
- Glass vials serve better option over PP microcentrifuge tubes for storing biological samples.



Abbreviations used: UFLC: Ultra Flow Liquid Chromatography; LC: Liquid Chromatography; MS: Mass spectrometry; AUC: Area Under Curve.

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DOI: 10.4103/0973-1296.185719

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INTRODUCTION

Liquid chromatography (LC) has been the most widely preferred and used separation technique among chromatographic methods and it has evolved tremendously since inception.[1] Utility of LC in different fields of applied and biological sciences has greatly been appreciated. Its wide acceptability is due to ability to analyze a wide range of molecules (biological and synthetic), with high sensitivity and precision.

Plant-based constituents are commonly being detected and quantified using LC-based methods.[2-7] An important step during this procedure is extraction, where the compound of interest is targeted using a particular extraction method.[8,9] Prepared extracts are then stored until further analysis. At present, research has been concentrated on optimization of extraction methods, particularly on solvents used for extraction, time for extraction, and other parameters are being studied.[8,9] Our recent studies on reversed phase ultra-flow LC (UFLC) analysis of newly recorded species *Achyranthes coynei* from Karnataka State, India,[5] implicated us toward such unnatural peaks emerging during the analysis.

An extensive literature survey showed only a handful of articles dealing on contamination issues. Wherein, bis (2,2,6,6-tetramethyl-4-piperidyl) sebacate, commonly known as tinuvin 770, has been reported as a

Cite this article as: Kshirsagar PR, Hegde H, Pai SR. Storing of extracts in polypropylene microcentrifuge tubes yields contaminant peak during ultra-flow liquid chromatographic analysis. Phcog Mag 2016;12:S303-6.

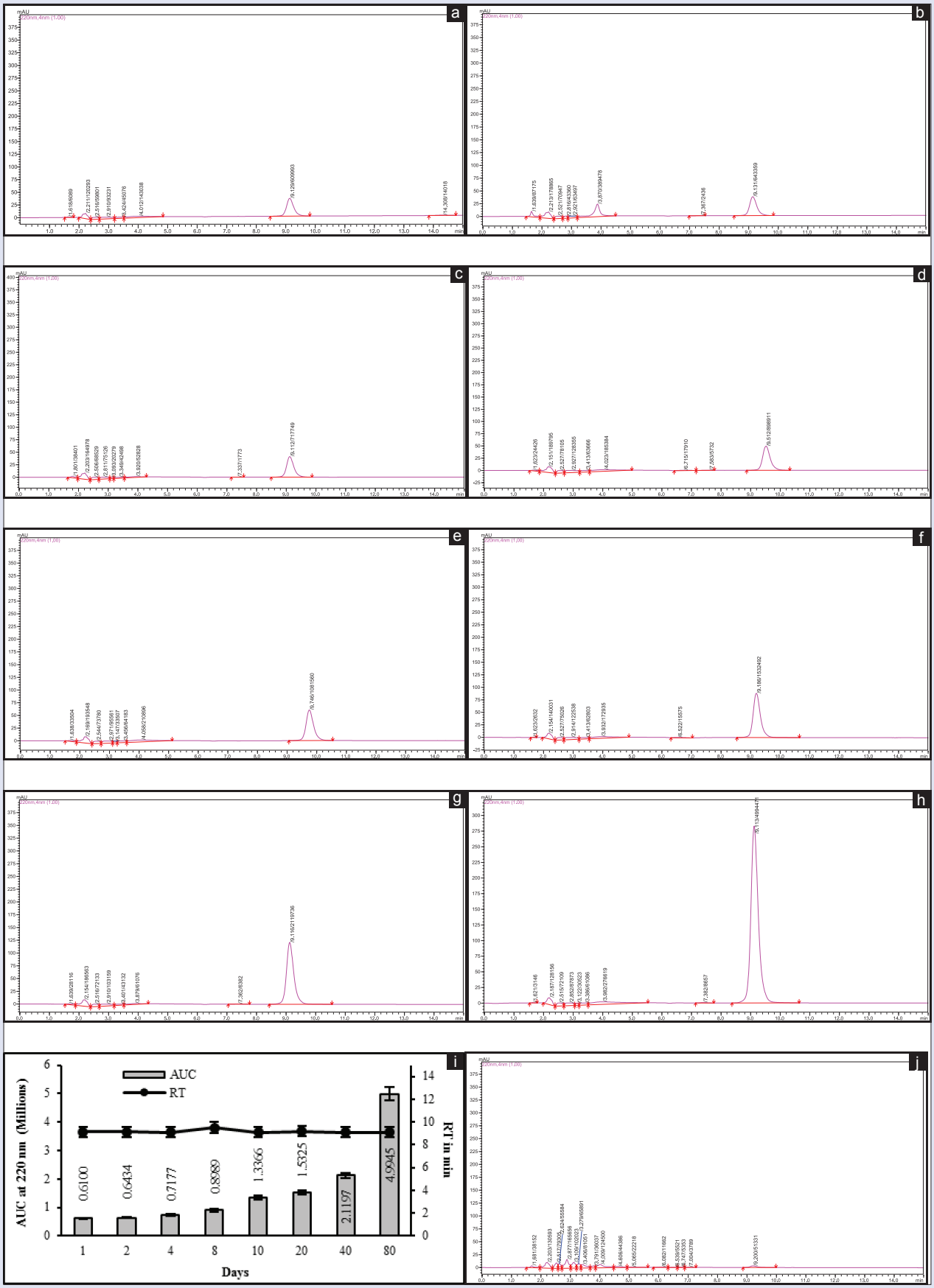

Figure 1: High-performance liquid chromatography profile of methanol stored in polypropylene microcentrifuge tubes at: (a) 1 day (b) 2 days (c) 4 days (d) 8 days (e) 10 days (f) 20 days (g) 40 days (h) 80 days and (i) histogram with area under curve and retention time of contaminant peak at different time intervals: (j) reversed phase-high performance liquid chromatography profile of methanol in glass vial stored for 10 days

major contaminant in a LC-mass spectrometry (MS)/MS proteomics experiment.[10] In the early 80's, this compound had been identified as ultraviolet stabilizer.[11] It is commonly used in the production of plastics, such as polypropylene and polystyrene.[12] Previous studies indicate that tinuvin 770 can leach from polypropylene tubes and interfere with common laboratory procedures, as well as have toxic effects on laboratory animals.[10,12-15] Thus, a study was designed to understand the effect of storage in polypropylene microcentrifuge tubes and glass vials during high-performance liquid chromatography analysis.

MATERIALS AND METHODS

Sample extraction

One ml of methanol was placed in polypropylene microcentrifuge tubes (PP material, Autoclavable) and glass vials (borosilicate) separately for 1, 2, 4, 8, 10, 20, 40, and 80 days intervals stored at −4°C. The UFLC analyses were performed immediately after completion of 80 days of the sample.

Ultra-flow liquid chromatographic analysis

The UFLC analysis was performed on Shimadzu chromatographic system (Model No. LC-20AD) consisting of a quaternary pump, manual injector, degasser (DGU-20A5), and a dual absorbance diode array detector (SPD-M20A). Chromatographic peak was achieved on a Waters 250–4.6 mm, 4 µ, Nova-Pak C18 column. A mobile phase consisting of methanol:water (70:30) was used for separation. A 20 µl injection volume, 1 ml/min flow rate, and 15 min analysis time were set for the analysis. The built-in LC-solution software system was used for data processing.

RESULTS AND DISCUSSION

An un-identified peak was observed in all injections of methanol stored in polypropylene microcentrifuge tubes during the UFLC run. This un-identified peak was termed as contaminant. The contaminant peak was prominent, sharp detectable at 9.176 ± 0.138 min. Alternatively, there were other peaks observed during the run, however none of them were characteristically compatible and comparable to the one recognized at this retention time (RT). Further, it is obvious that the obtained sharp peak would easily have a compatibility issue between peaks of interest during any phyto-chemical/biochemical analysis.[16]

Figure 1a-h depicted the UFLC profile of eight separate injections made each for the representative day selected, whereas Figure 1i showed the correlation between area under curve (AUC) and different time intervals (days). As time period of storage increased, there was a marked increase in the area of the contaminant peak, with no difference observed in RT of the peak. Figure 1j showed the UFLC profile of methanol stored in glass vial for 10 days, wherein there was a small peak observed at 9.200 min. This peak of methanol stored in glass vial for 10 days (AUC: 51331) had ~91.60% lesser area than that of the 1st day methanol stored in polypropylene microcentrifuge tubes (AUC: 609993).

Figure 2 showed representative data for 80th day sample. The three-dimensional contour view of the chromatogram showed position of the peak with respect to RT (9.113 min) and wavelength (220.38 nm) alongside its intensity (300 mAU) [Figure 2a]. Spectral data with λ max (220.38 nm) for the peak were also recorded [Figure 2b and c]. It was apparent from the purity index profile that there were no impurities detected (purity index 1.000) with a single point threshold of 0.999.

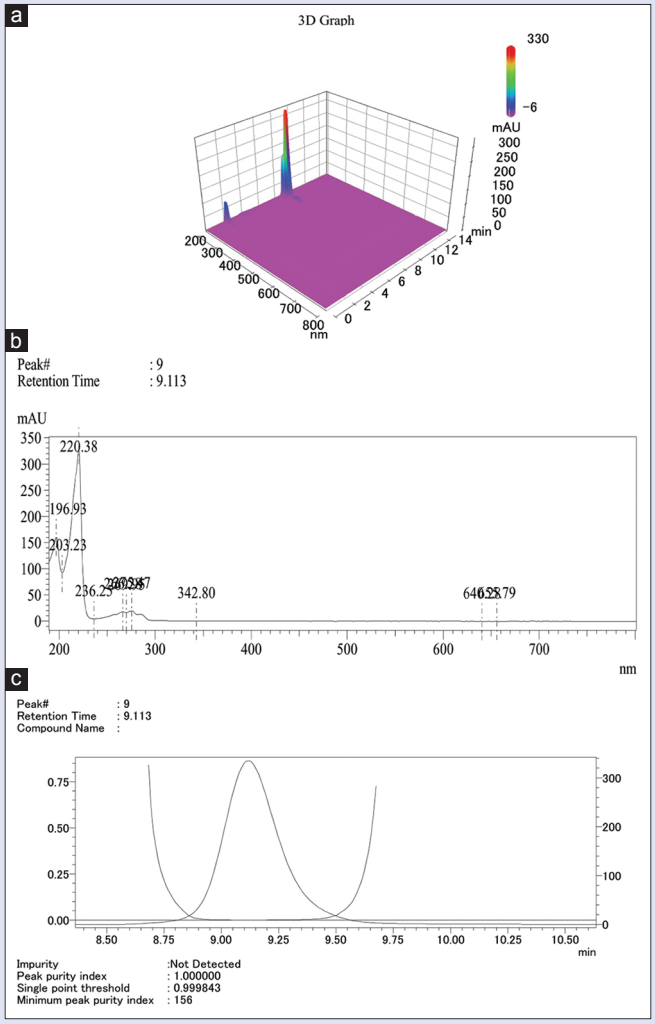

Figure 2: Representation of 80th day sample: (a) Three-dimensional contour view of contaminant peak: (b) Spectral data (λ max 220.38 nm); (c) Peak purity index

CONCLUSION

Consequently, it is evident that long-time storage of biological samples, especially prepared in methanol will produce a contaminant peak during UFLC analysis. Thus, it is advisable to run a solvent used for extraction, stored for equal number of days as that of extracts as day-blank to identify the contaminant on a set chromatographic condition. Even, preferably, it is recommended to avoid storing of such samples in polypropylene microcentrifuge tubes, which otherwise may mislead in terms of reporting an unnatural compound by researchers.

Acknowledgments

The authors are indebted to Officer-in-Charge, RMRC, Belgaum, and Indian Council of Medical Research, New Delhi, for providing necessary facility. The authors are also thankful to Mr. Venkatesh Millanhatti, for his help during the study. SRP is also thankful to SERB, DST, New Delhi, for providing financial assistance during the work.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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