Bulletin of Biological and Allied Sciences Research
ISSN: **2521-0092**
www.bbasr.org
DOI: https://doi.org/10.64013/bbasr.v2026i1.141
*Bull. Biol. All. Sci. Res., Volume, **11**: 141*
Original Research Article ***Open Access***

REPEATED PRESSURE-RELATED CHROMATOGRAPHIC FAILURE DURING SALIVARY CORTISOL ANALYSIS BY LC-HRMS/MS: A LABORATORY CASE REPORT AND HYPOTHESIS-DRIVEN ROOT CAUSE ASSESSMENT

UMRAO SR*, ELANGOVAN S, THARAMMAL MS, QURESHI MR, MOHAMMED SHS, ALAM MM, ASHRAF F, AMIN FU, KHAN MM

Life Core Private Clinic LLC, Royal Group Headquarters (7th Floor), Al Salam Street, PO Box 63811, Abu Dhabi, United Arab Emirates
**Correspondence Author Email Address: dr.sushma@royalgroupuae.com*

(Received, 20th January 2026, Accepted 27th October 2026, Published 12th November 2026)

Abstract *Liquid chromatography tandem mass spectrometry (LC-MS/MS) is the reference standard method for quantitatively measuring salivary cortisol, largely because of its high analytical specificity and sensitivity. However, saliva is a complex biological matrix that may compromise chromatographic robustness during routine testing. Here, we report a laboratory case of system back-pressure exceedance, chromatographic deterioration, and analytical column collapse during routine LC-HRMS/MS analysis of salivary cortisol using a commercially available sample preparation and analysis kit. Two columns used in assay analysis failed consecutively. Both columns were subjected to the same working conditions. Factors such as how the samples were injected, chromatograms, and the behaviour of the system were analyzed. Possible causes of failures were also evaluated based on the pressure and behaviour of the columns. The team also evaluated possible causes based on column pressure and behaviour. The review indicated that the matrix caused the failure. Poorly removed salivary mucins and other particles clogged the columns. Flow restriction caused retention time to vary. The columns became worn out because the phase failed to remain permanently fixed to the column. Failures occurred in two columns and suggested a limitation of the method regarding matrix interaction with LC-MS/MS. This occurred mainly when high-viscosity saliva samples were analyzed using UHPLC columns. Restrictions were noted particularly at the column inlet. It was recommended that columns be well protected and that preventive maintenance be performed to reduce column failures and loss of phase integrity. This is especially true in laboratories that analyze salivary cortisol using LC-MS/MS. The report presents a case study illustrating the types of column failures caused by matrix limitations in LC-MS/MS analysis.*

[*Citation:* Umrao, S.R., Elangovan, S., Tharammal, M.S., Qureshi, M.R., Mohammed, S.H.S., Alam, M.M., Ashraf, F., Amin, F.U., Khan, M.M. (2026). Repeated pressure-related chromatographic failure during salivary cortisol analysis by LC-HRMS/MS: A laboratory case report and hypothesis-driven root cause assessment. *Bull. Biol. All. Sci. Res.* **11***: 141*. https://doi.org/10.64013/bbasr.v2026i1.141]
Keywords*:* *LC-HRMS/MS; Orbitrap; PRM; salivary cortisol; column fouling; back-pressure; matrix effects; method robustness; sample preparation; root-cause analysis*

1. Introduction

Salivary cortisol is an increasingly common noninvasive measure of HPA axis function in clinical and research settings. It is a free fraction measure, is not affected by cortisol-binding globulin, and offers particular advantage for assessment of the diurnal cortisol rhythm and the stress response as well as for screening for Cushing's syndrome (Raff, 2009; Mezzullo et al., 2016). Measuring the free fraction of cortisol can be challenging because residual food, blood, and topical corticosteroids can all contaminate the sample before it is collected. LC-MS/MS is considered the reference method for steroid hormone analysis because of its high analytical specificity and sensitivity when compared to immunoassays (Keevil, 2016; Vogeser and Seger 2008). However, saliva as an analytical matrix presents challenges because of variability between individuals in viscosity, protein content, mucin burden, and particulates, which can all alter the retention of analytes over time (Taylor, 2005; Chiappin et al., 2007). Further, significant alterations to long-term matrix performance of a method cannot always be captured during short-term validation studies (Matuszewski et al., 20023; Van Eeckhaut et al., 2009). It is also well described in the bioanalytical LC-MS/MS literature that use of sub-2 µm UHPLC columns with un-purified biological matrices can lead to rapid column fouling and failure (Rodríguez-Cabo et al., 2009; Zuvela et al., 2019). The economic and operational consequences of column failure in a routine clinical laboratory are significant. Although known risks of column failure exist, there are relatively few documented cases of complete failure using commercially available salivary cortisol assay kits under routine conditions.

Studies like this are crucial because they show the gap in controlled method-validation finding and what actually happens in a working lab, and because they give other laboratories a practical framework for

dealing with the same problems. Here we describe a reproducible pattern of back-pressure exceedance and analytical column failure that we encountered during routine LC-HRMS/MS analysis of salivary cortisol in an ISO 15189-accredited clinical laboratory. Here we describe a repeatable pattern of excessive back-pressure and analytical column failure that showed up during routine LC-HRMS/MS testing of salivary cortisol in our ISO 15189-accredited clinical laboratory. This report walks through what we observed when the failures occurred, why we think they happened based on sound chromatographic reasoning, and what laboratories can do to manage them. We see this as a matrix-method interaction issue rather than a fault of any specific product, and we hope it helps anyone considering or already running LC-MS/MS salivary cortisol testing.

2. Materials and Methods

2.1 Setting

Analyses were performed in the clinical mass spectrometry section of LifeCore Private Clinic, Abu Dhabi, UAE - an ISO 15189-accredited clinical laboratory. Salivary cortisol testing was performed on routine patient specimens referred for assessment of HPA axis function.

2.2 Instrumentation

Analyses were performed using a Thermo Scientific Q Exactive Plus hybrid quadrupole Orbitrap high-resolution mass spectrometer coupled to a UHPLC system (Thermo Fisher Scientific, Bremen, Germany) equipped with a heated electrospray ionisation (HESI-II) source operated in positive ion mode. Chromatographic separation was coupled online to high-resolution mass spectrometric detection. Data acquisition and processing were performed using Xcalibur software (Thermo Fisher Scientific).

2.3 Chromatographic Conditions

Chromatographic separation was achieved on a reversed-phase analytical column Poroshell 120 EC-C18 (50 × 2.1 mm, 1.9 µm). The mobile phase consisted of Mobile Phase M1 (Pump A) and Mobile Phase M2 (Pump B), supplied with the kit. delivered under gradient conditions as specified by the kit manufacturer. The flow rate was 0.400 mL/min, the column temperature was 45°C, and the injection volume was 15 µL. The total run time per injection was 7.50 min.

2.4 Mass Spectrometric Conditions

Quantification was performed in parallel reaction monitoring (PRM) mode using the transitions m/z 363.2 → 121.1 (cortisol, quantifier) and m/z 363.2 → 97.0 (cortisol, qualifier). The deuterated internal standard cortisol-d4 was monitored at m/z 367.2 → 121.1. Mass spectrometric source parameters were optimized to maximize the signal response of low-level standard solutions.

2.5 Sample Collection and Pre-analytical Handling

Saliva samples were collected using Salivette® Cortisol or equivalent saliva collection devices. Subjects were instructed to remain fasting and avoid intense physical activity for at least 2 h before sampling. Ten minutes before collection, participants rinsed their mouths with cold water, and the collection time was recorded. Samples were centrifuged immediately after collection to improve stability, and the clarified saliva was subsequently processed for LC-HRMS/MS analysis according to the manufacturer's protocol.

2.6 Sample Preparation

Sample preparation was performed using the Eureka Cortisol and Cortisone in Saliva by LC-MS/MS kit (REF LC91110; Eureka S.r.l., Lab Division, Italy) according to the manufacturer's instructions. Briefly, 500 µL of saliva sample was transferred into an Eppendorf tube and mixed with 10 µL of Internal Standard Solution (Reagent A) and 20 µL of Clarifying Solution (Reagent B). The mixture was vortexed for 20 seconds and then centrifuged at 14,000 rpm for 10 minutes. A 300 µL aliquot of the supernatant was transferred to a fresh Eppendorf tube, combined with 100 µL of Stabilization Solution (Reagent C), and vortexed for a further 20 seconds. At the end the extract was kept in an autosampler vial, and injected with 15 µL for LC-HRMS/MS examination.

2.7 System Suitability and Performance Monitoring

System back pressure was monitored during analytical runs to ensure it stayed within both the manufacturer's specified and the method defined limits.

2.8 Column History

Two different analytical columns were used during the investigation. Each column was dedicated for the analysis of salivary cortisol. Column A was installed on 08 September 2025 and expired after ~15 injections. Column B was installed on 16 October 2025 and also failed after ~15 injections. Columns A and B were brand new when installed, and we ran them under the manufacturer's recommended conditions for pressure, temperature and mobile phase.

3. Results

3.1 Increased Backpressure

Both the columns used for this analysis (A and B) started having problems after ~10 injections. Problems with Column A (installed 08 September 2025) started when the instrument raised communication errors between the pumps (Figure 1). Problems with Column B (installed 16 October 2025) started with leak detection alarms (Codes 33 and 34) and eventually the method abort was raised (Figure 2). Since the same issues were observed with two different columns, we believed it was an issue with the LC-MS/MS system and its associated hardware.



		Date	Time	Retention Time	Device	Message
1		08/09/2025	1:19:30 PM +04:00		SamplerModule	Driver has disconnected.
2		08/09/2025	1:19:20 PM +04:00		ColumnComp	Driver has disconnected.
3		08/09/2025	1:19:20 PM +04:00		PumpModule	Driver has disconnected.
4		08/09/2025	1:19:19 PM +04:00		PumpModule	Error reading from VPUMP @ USB-0315592. A device attached to the system is not functioning.
5		08/09/2025	1:19:19 PM +04:00		SamplerModule	Error reading from VAS @ USB-0315639. A device attached to the system is not functioning.
6		08/09/2025	1:19:19 PM +04:00		ColumnComp	Error reading from VTCC @ USB-6504426. A device attached to the system is not functioning.
7		08/09/2025	10:25:24 AM +04:00	-1.395		Running sequence queue intercepted by user Sampathkumar.
8		08/09/2025	10:02:52 AM +04:00		SamplerModule	Temperature control is on but not all doors are closed. Close both doors or temperature control will shut down in 20.0 minutes. Code 9032.
9		08/09/2025	10:01:40 AM +04:00		SamplerModule	Temperature control is on but not all doors are closed. Close both doors or temperature control will shut down in 20.0 minutes. Code 9032.

Figure 1. Communication error event (08 September 2025)

1738		16/10/2025	9:33:33 AM +04:00			Instrument method aborted (because an abort or fatal error has occurred).
1739		16/10/2025	9:33:33 AM +04:00	2.831	SamplerModule	Leak detected. Code 34.
1740		16/10/2025	9:32:53 AM +04:00	2.164	SamplerModule	Leak detected - eliminate within approx. 30 seconds. Code 33.
1741		16/10/2025	9:32:23 AM +04:00	1.664	SamplerModule	Leak detected - eliminate within approx. 60 seconds. Code 33.

Figure 2. Leak detection event (16 October 2025)

3.2 Clarification of Pump Error Messages

During the failure event, the pump recorded two pressure-related error messages. The first indicated that the maximum pressure limit had been reached and automated a system shutdown. Shortly after, the second message, indicating a low pressure condition or leak, was generated. The second message is likely a result of the drop in pressure after the system shut down. The sequence of events is illustrated in Figure 2.

3.3 Chromatographic Deterioration

The analysis of the cortisol and the internal standard chromatograms indicated a deterioration of peak shape during analysis. The peaks became broad and had a loss of symmetry with increased baseline disturbance. Compared to the expected chromatographic profile, both the cortisol peak and the internal standard cortisol-d4 peak showed retention time increases and eluted at approximately 3.49 and 3.70 minutes, respectively. These results are consistent with decreased column performance and increased backpressure.

3.4 Internal Standard and Quantitative Performance

The retained internal standard (cortisol-d4) and the analyte peak of cortisol indicated retention time drift. The retention time drift was due to a chromatographic cause and not a mass calibration drift. Internal quality control values indicated that imprecision increased and a negative bias at low values was observed during the final stages before the column failure. These values, in conjunction with the other observed deteriorations, chromatographic performance and column integrity, indicated failure of the chromatographic system.

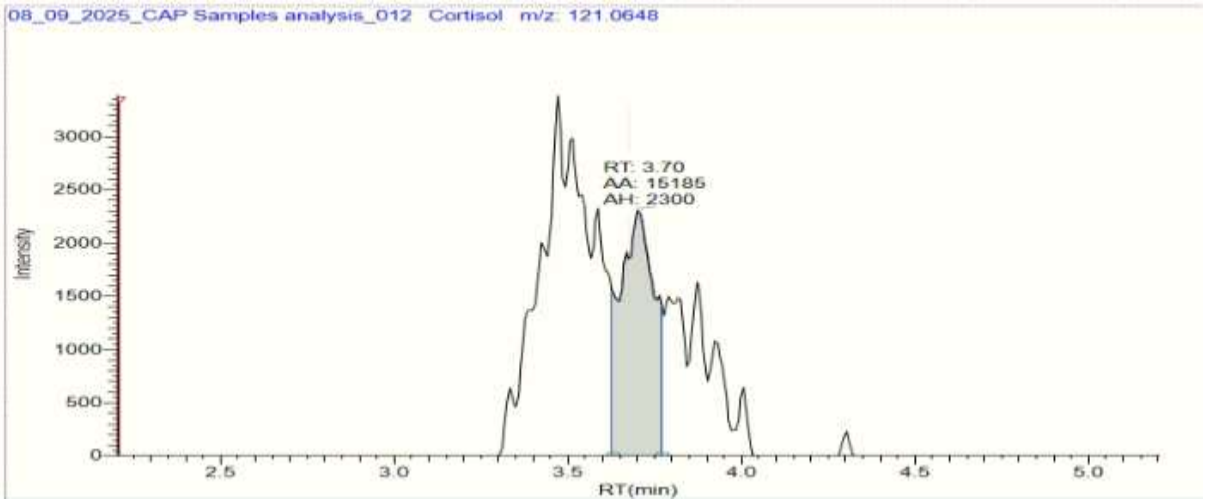

Figure 3 Representative chromatograms of Cortisol

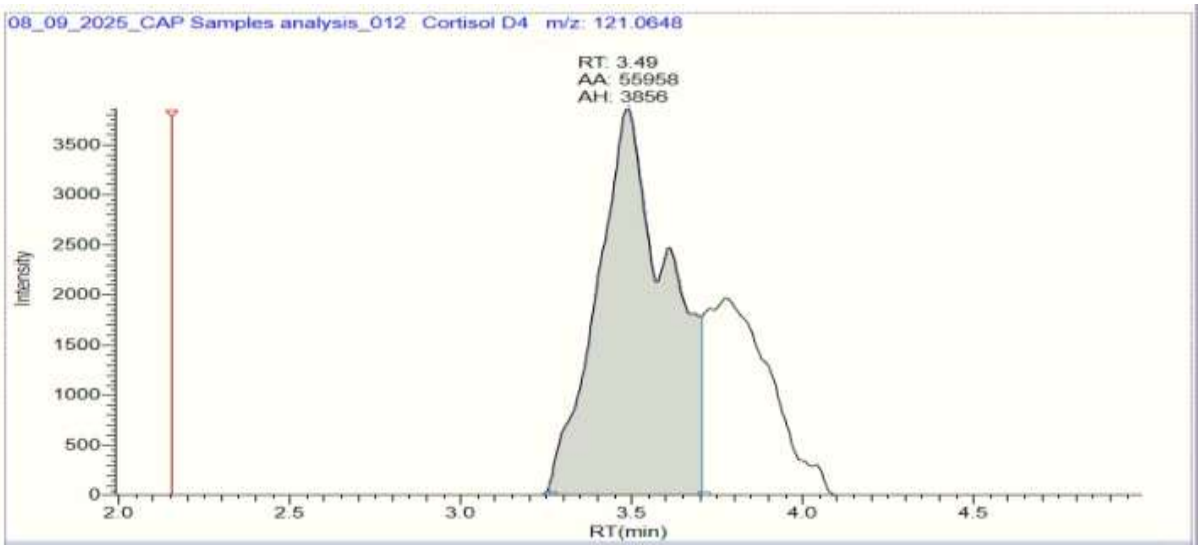

Figure 4 Representative chromatograms of Cortisol-D4

3.5 Postmortem Column Inspection

No post-mortem visual or microscopic examination, column disassembly, back-flushing experiments, surface characterization, or chemical analysis of retained samples was performed. As a result, the position and nature of the flow-restrictive materials could not be definitively established.

3.6 Impact on Operations

Failure of two analytical columns during the study period led to disruptions of routine operations in the laboratory and replacement of the failed columns. Analytical testing was delayed while corrective actions were being implemented. No resultant patient data were released due to the instrument errors. Samples associated with the interrupted analytical sequence were re-analyzed upon restoration of stable assay performance and system suitability.

4. Root Cause Analysis

A root cause analysis was performed to understand potential mechanisms of failure based on the evidence available. Each hypothesis was assessed by reviewing chromatographic data, column inspection findings, and the relevant analytical chemistry literature. The results are summarized in Table 1.

Table 1. Hypothesis-driven root-cause analysis of the observed failure pattern

Hypothesis	Expected evidence if true	Observed evidence	Assessment
Isolated column defect	Failure confined to a single analytical column, with subsequent columns operating normally under comparable conditions.	A similar pressure-related and chromatographic failure pattern occurred with two separate, newly installed assay-dedicated columns operated using the same analytical workflow.	**Less consistent with the observations** Recurrence with two columns makes an isolated single-column defect less likely; however, it was not formally excluded experimentally.
General instrument malfunction, including pump, injector, or seals	Comparable pressure abnormalities or diagnostic faults occurring independently of the salivary cortisol assay and potentially affecting other analytical methods on the same instrument.	Pressure-related interruption occurred during the salivary cortisol workflow, whereas other assays performed on the same instrument did not show a comparable recurrent pattern.	**Less consistent with the observations but not formally excluded** The assay-associated recurrence does not strongly support a generalized instrument malfunction.
Matrix-associated flow restriction	Deterioration in pressure and/or chromatographic performance during repeated injections of prepared biological-matrix extracts, with recurrence under comparable analytical conditions.	Repeated pressure-related interruptions and chromatographic deterioration occurred during sequential analysis of prepared saliva extracts with two analytical columns. However, the location and composition of any restriction were not directly determined.	**Plausible and most consistent with the observed pattern; not directly confirmed** Matrix-associated flow restriction is considered a working hypothesis rather than an established mechanism.

Incomplete removal of matrix constituents during sample preparation	Detectable residual protein, mucin, particulate material, or other matrix components after clarification, with reduced matrix burden and improved chromatographic robustness following additional clean-up.	The manufacturer-specified clarification procedure was used without an additional filtration or SPE step. Residual protein, mucin, or particulate burden was not measured, and no controlled comparison with enhanced sample preparation was performed.	**Potential contributing factor; not experimentally demonstrated.** Additional matrix removal requires prospective evaluation.
Column format/particle size contributing to susceptibility to flow restriction	Increased susceptibility to additional hydraulic resistance during repeated analysis of complex biological extracts, with improved robustness demonstrated using an alternative column configuration or protective device.	Both affected analytical columns used 1.9 µm particles. No controlled comparison with alternative particle sizes, column formats, guard columns, or in-line filters was performed.	**Possible contributing factor; not independently established.** The available data do not demonstrate that the sub-2 µm column format itself caused the failure.

Putting it all together, what we saw fits best with the sample matrix gradually raising the resistance to flow through the system as prepared saliva extracts were injected again and again. Two things may have played a part: matrix components that were not fully removed, and the use of sub-2 µm columns. That said, we never pinpointed where the restriction was located, and we did not characterize whatever material might have been retained. For that reason, we treat both explanations as working hypotheses, not as causes we have confirmed experimentally.

5. Discussion

LC-MS/MS is the most popular technique for the quantification of steroid hormones. This technique includes the use of isotopically labelled internal standards for quantification, offers high specificity and shows minimal cross-reactivity (Keevil, 2016; Vogeser and Seger 2008), thus making it attractive for adoption in clinical laboratories. The current case illustrates that during the day-to-day analysis, systematic variations introduced by the biological matrices may affect the chromatography more than the method validation studies performed in the controlled lab environment. Of all the biological matrices, saliva presents the most difficulty due to its viscosity, protein content, mucins, cells, and particulate matter (Taylor, 2005; Chiappin et al., 2007). Leaving behind matrix constituents during sample preparation may adversely affect chromatography. In this work, however, the burden of protein, mucin, and particulate matter was not directly assessed and, therefore, should be considered a possible factor, not a demonstrated factor. Retention time drift and peak broadening are consistent with increased flow resistance in the chromatographic system. However, since other possible failure modes (e.g., physically examining and backflushing the columns) were not performed, the location and nature of the failure are unknown. So, for now, we see matrix-related flow restriction as a reasonable working hypothesis, not a mechanism we have confirmed.

Our sample-preparation workflow stopped at the clarification procedure specified by the manufacturer, with no extra filtration or solid-phase extraction (SPE) step afterward. Other ways of removing more of the matrix, such as a second clarification, filtration, higher centrifugal force, or SPE, could be worth testing in future studies. That said, we did not compare any of these approaches in a controlled way here, so our data cannot tell us whether they would have prevented the pressure-related failures we saw. Also, any change to the commercial sample-preparation procedure would need proper analytical verification before being adopted.

5.1 Implications for Method Validation

These observations reinforce the importance of extended robustness testing as part of method validation for LC-MS/MS assays on viscous biological matrices. Standard validation parameters (accuracy, precision, linearity, recovery, lower limit of quantification) are necessary but not sufficient. We recommend that validation studies for saliva-based LC-MS/MS methods also include: (a) extended sequence testing over at least 200-300 injections of authentic clinical samples; (b) continuous back-pressure monitoring with pre-defined trend thresholds; (c) retention-time stability assessment across the same extended sequence; and (d) explicit evaluation of the effect of additional clean-up steps (filtration, SPE) on column life, separately from their effect on accuracy and precision.

5.2 Implications for Routine Operation

For laboratories already operating salivary LC-MS/MS assays, we recommend proactive monitoring of system back-pressure as a leading indicator of column health, analogous to the role of internal quality control for analytical accuracy. If back-pressure keeps increasing and reaches around 120–150% of its baseline during a series of injections, it may be a sign that the column needs attention. Washing the column or replacing the guard column at this stage can help prevent a complete failure. Retention-time shifts, changes in peak shape, and lower signal-to-noise ratios should also be monitored,

as they can indicate developing problems before they become more serious.

6. Mitigation and Practical Recommendations

Based on the observations and root-cause analysis, we recommend the following practices for laboratories operating or implementing LC-MS/MS assays on saliva or other viscous biological matrices:

Enhanced sample preparation. An extra filtration step can be added to the kit's standard protocol to remove any remaining particles. One simple option is to filter the sample through a 0.22 µm syringe filter or a 96-well filter plate after protein precipitation. If further removal of matrix components is needed, mixed-mode solid-phase extraction (SPE) can be used, although it adds extra steps and makes the overall procedure more complex.

Column protection. Use an in-line guard column with the same chemistry as the analytical column. Guard columns are significantly less expensive than analytical columns and can be replaced on a pressure-trend-driven schedule, protecting the analytical column from most of the matrix load. Control of injection parameters. Adjust injection volume to a smaller value, if possible, while maintaining required sensitivity of the assay. Evaluate a two-fold dilution strategy, in combination with a larger volume injection of the extract, for high matrix samples. This can help reduce the absolute matrix mass per injection while maintaining required sensitivity of the assay. Preventive maintenance and performance monitoring. Carry out routine column washes using a solvent sequence at defined intervals. Continuously monitor back pressure and retention times and define threshold action levels (e.g. +30% baseline). Local method verification. Thoroughly evaluate vendor validation data. If vendors do not validate saliva as a test matrix, perform comprehensive evaluation using a defined sample set that is representative of the assay's routine workload.

7. Limitations

This study has some limitations that should be considered when interpreting the findings. First, the observations were made during routine clinical use rather than under controlled experimental conditions. Details such as the number of injections before column failure and the rate at which back-pressure increased were obtained from instrument logs after the events occurred, rather than from a predefined study protocol. Second, the commercial kit contains proprietary components, so the authors could not fully describe the chemistry involved in sample preparation. Finally, the column was examined after failure using visual inspection and back-flush testing only. More detailed investigations, such as electron microscopy or surface chemical analysis, were not carried out and could have provided further insight into the causes of column deterioration. Fourth, the report is based on experience at a single laboratory, and the generalisability of the findings to other instrument platforms or other saliva collection devices requires confirmation by other centres. Finally, a formal controlled comparison with and without additional filtration was not undertaken prospectively; the recommendation in favour of additional clean-up is based on mechanistic reasoning and the published literature rather than on a direct experimental comparison in this laboratory.

8. Conclusion

Progressive back-pressure exceedance and sequential column failure during routine LC-HRMS/MS analysis of salivary cortisol were observed in association with a commercial analytical kit employing a protein-precipitation sample-preparation workflow. Our root-cause analysis points to an interaction between the sample matrix and the method: when particulates and proteins are not removed thoroughly enough, they gradually build up and foul the inlet of sub-2 µm UHPLC columns. We offer this report as a practical example of how far controlled method validation can sit from the day-to-day robustness a method needs in real laboratory use, and as a framework for other laboratories that run into similar problems. Our main recommendations are to validate methods with the matrix in mind, to monitor back-pressure proactively, and to add sample clean-up steps beyond those that come with the kit workflow.

References

Chiappin, S., Antonelli, G., Gatti, R., & De Palo, E. F. (2007). Saliva specimen: A new laboratory tool for diagnostic and basic investigation. *Clinica Chimica Acta*, 383(1–2), 30–40. https://doi.org/10.1016/j.cca.2007.04.011

Keevil, B. G. (2016). LC–MS/MS analysis of steroids in the clinical laboratory. *Clinical Biochemistry*, 49(13–14), 989–997. https://doi.org/10.1016/j.clinbiochem.2016.04.009

Matuszewski, B. K., Constanzer, M. L., & Chavez-Eng, C. M. (2003). Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC–MS/MS. *Analytical Chemistry*, 75(13), 3019–3030. https://doi.org/10.1021/ac020361s

Mezzullo, M., Fanelli, F., Fazzini, A., Gambineri, A., Vicennati, V., Di Dalmazi, G., Pelusi, C., Mazza, R., Pagotto, U., & Pasquali, R. (2016). Validation of an LC–MS/MS salivary assay for glucocorticoid status assessment: Evaluation of the diurnal fluctuation of cortisol and cortisone and of their association within and between serum and saliva. *Journal of Steroid Biochemistry and Molecular Biology*, 163, 103–112. https://doi.org/10.1016/j.jsbmb.2016.04.012

Raff, H. (2009). Utility of salivary cortisol measurements in Cushing's syndrome and adrenal insufficiency. *Journal of Clinical*

Endocrinology & Metabolism, 94(10), 3647–3655. https://doi.org/10.1210/jc.2009-1166

Rodríguez-Cabo, T., Rodríguez, I., Ramil, M., & Cela, R. (2019). Sample clean-up strategies for complex biological matrices in LC–MS/MS analysis. *Journal of Chromatography B*, 1125, 121706. https://doi.org/10.1016/j.jchromb.2019.121706

Taylor, P. J. (2005). Matrix effects: The Achilles heel of quantitative high-performance liquid chromatography–electrospray–tandem mass spectrometry. *Clinical Biochemistry*, 38(4), 328–334. https://doi.org/10.1016/j.clinbiochem.2004.11.007

Van Eeckhaut, A., Lanckmans, K., Sarre, S., Smolders, I., & Michotte, Y. (2009). Validation of bioanalytical LC–MS/MS assays: Evaluation of matrix effects. *Journal of Chromatography B*, 877(23), 2198–2207. https://doi.org/10.1016/j.jchromb.2009.01.003

Vogeser, M., & Seger, C. (2008). A decade of HPLC–MS/MS in the routine clinical laboratory—Goals for further developments. *Clinical Biochemistry*, 41(9), 649–662. https://doi.org/10.1016/j.clinbiochem.2008.02.017

Žuvela, P., Skoczylas, M., Liu, J. J., Baczek, T., Kaliszan, R., Wong, M. W., & Buszewski, B. (2019). Column characterization and selection systems in reversed-phase high-performance liquid chromatography. *Chemical Reviews*, 119(6), 3674–3729. https://doi.org/10.1021/acs.chemrev.8b00246

Statements and Declarations

Ethics Approval

This report describes laboratory analytical performance and equipment failure. No patient-identifiable data are presented. Institutional ethics approval was not required for this analytical case report under institutional policy.

Data Availability

The corresponding author can provide the data upon reasonable request, subject to patient confidentiality constraints.

Author Contributions

SRU conceived the investigation, led the root-cause analysis, and drafted the manuscript. SE, MST, MRQ, SHSM, MMA, FA, FUA, and MMK contributed to the analytical investigation, data review, and critical revision of the manuscript. All authors approved the final version.

Declaration of Generative AI and AI-assisted Technologies in the Writing Process

During the preparation of this work, the author(s) used ChatGPT (OpenAI) to assist with language refinement and restructuring for clarity. After using this tool, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

Funding

This study did not receive any particular grant from any funding agency, whether in a public, private, or non-profit organization.

Informed Consent Statement

Not applicable. This study did not involve human participants.

Conflicts of Interest

The authors confirm that they have no conflicts of interest.

Consent for publication

All the authors agree to publish the manuscript.

Acknowledgment

Not applicable.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, Creative Commons Attribution-NonCommercial 4.0 International License, © The Author(s) 2026