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Page: 01-10

LC–MS ANALYSIS OF MAJOR ALKALOIDS IN HOMOEOPATHIC MOTHER TINCTURE OF JUSTICIA ADHATODA

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ABSTRACT

Background: *Justicia adhatoda* L. (syn. *Adhatoda vasica* Nees) is a respiratory remedy whose activity is attributed to quinazoline alkaloids. Its hydro-alcoholic mother tincture (Ø) is the starting material for homoeopathic dilutions, but LC–MS data on the tincture are scarce. Aim: To characterise the major alkaloids of the mother tincture by LC–MS. Methods: One tincture sample was analysed on 25 September 2026 (5 µL) on an LCMS-8045 RX triple-quadrupole system by full-scan acquisition (20 positive/negative events) and multiple reaction monitoring (MRM) of eleven analytes with quantifier and qualifier transitions. Identification was assessed from retention-time (RT) agreement and ion ratio. Relative peak-area shares were used as signal proportions, not as estimates of compound amounts. Results: Full-scan base peaks at *m/z* 205, 189, 219 and 203 coincided with the RT windows of vasicinol, vasicine, vasicoline and vasicinone. MRM signals were observed for all nine targeted alkaloids, but two identifications were tentative. Vasicinone gave the strongest signal (43.5% of summed alkaloid peak area), followed by vasicine (28.7%), vasicoline (14.8%) and vasicinol (9.0%); together these accounted for 96.0% of the alkaloid signal. Orientin was not confirmed. Conclusion: The signal profile was dominated by vasicinone and vasicine. Because detector response can differ among compounds and no calibration standards were used, peak-area shares must not be interpreted as relative amounts. Validated analysis with reference standards is needed.

KEYWORDS: *Justicia adhatoda*; mother tincture; homoeopathy; vasicine; vasicinone; LC–MS/MS; MRM.

INTRODUCTION

Justicia adhatoda L. (*Adhatoda vasica* Nees; Vasaka, Malabar nut; Acanthaceae) is a perennial shrub [4] used traditionally for cough, bronchitis and asthma [1,13]. Its aerial parts contain the quinazoline alkaloids vasicine and vasicinone, with deoxyvasicine, vasicinol and others [4,5,8]. Vasicine is reported to be bronchodilatory and respiratory-stimulant, and vasicinone bronchodilatory and anti-anaphylactic [8]; vasicinone also arises by auto-oxidation of vasicine [4]. The mucolytic drug bromhexine was developed from vasicine [2]. In homoeopathy, the hydro-alcoholic extract of the leaves (mother tincture, Ø) is the starting material for all dilutions, and a draft Indian standard requires a minimum alkaloid content as vasicine plus a chromatographic identity test [3]. Additional phytochemical and isolation studies have also examined *J. adhatoda* leaves and related *Adhatoda* species [12,14].

Rationale and need of the study

Published analytical work concerns mostly leaves, juices, extracts and Ayurvedic products [4,5,8,9]. Because every potency derives from the mother tincture, a selective profile of its alkaloids is needed to confirm their presence, judge their relative abundance and propose marker compounds.

Review of Literature

Vasicine and vasicinone have been quantified by HPLC–PDA [6], capillary electrophoresis (11 min) [7] and HPTLC [4,10]. A validated UPLC/Q-TOF-MS method for vasicine was reported as more sensitive and selective than TLC, HPLC and GC-MS [5], and LC–MS/MS has been used to profile leaf extracts [11]. RP-HPLC of marketed preparations found vasicine at about 1.5–1.8 g per 100 mL [9]. Other constituents, such as lupeol, stigmasterol and β -sitosterol, have also been quantified [15].

Research Gap

Existing methods target mainly vasicine and/or vasicinone [4,6,7,10]; minor alkaloids are rarely profiled together, and in the sources reviewed no LC–MS study of a *homoeopathic mother tincture* was found. The draft standard [3] relies on total alkaloids as vasicine and does not describe individual alkaloids.

Aim and Objectives

Aim: To identify and compare the major alkaloids in a homoeopathic mother tincture of *Justicia adhatoda* by LC–MS.

Objectives

1. To record the TIC and averaged full-scan spectra in positive and negative modes.
2. To screen nine alkaloids and two flavonoid C-glycosides by MRM.
3. To assess identification reliability (RT agreement, ion ratio).
4. To compare relative abundance and relate findings to the literature.

Hypothesis

H₀: The mother tincture contains no detectable amounts of the target alkaloids. **H₁:** It contains detectable quinazoline alkaloids, with vasicine and vasicinone as the major components.

MATERIALS AND METHODS

Study setting

Laboratory-based study using an LCMS-8045 RX triple-quadrupole LC–MS/MS system. The laboratory name and location were not documented in the study details available for this report. Data were acquired on 25 September 2026 (13:12:51) and processed on 28 September 2026 with LabSolutions Insight 4.2.168.1.

Study design

Exploratory, descriptive, qualitative and semi-quantitative analytical study of one mother tincture sample, with a single 5 µL injection.

Procedure

Full-scan acquisition used 12 positive (TIC(+))@2–@13) and 8 negative (TIC(–))@14–@21) events, followed by MRM of eleven analytes (Table 1). The supplied study record does not specify the sample manufacturer/batch and preparation, LC column, mobile-phase solvents and gradient, flow rate, column temperature, ion-source settings, or MRM collision/voltage settings. These parameters must be recovered from the original instrument method and sample records for reproducibility. The workflow is shown in Figure 1.

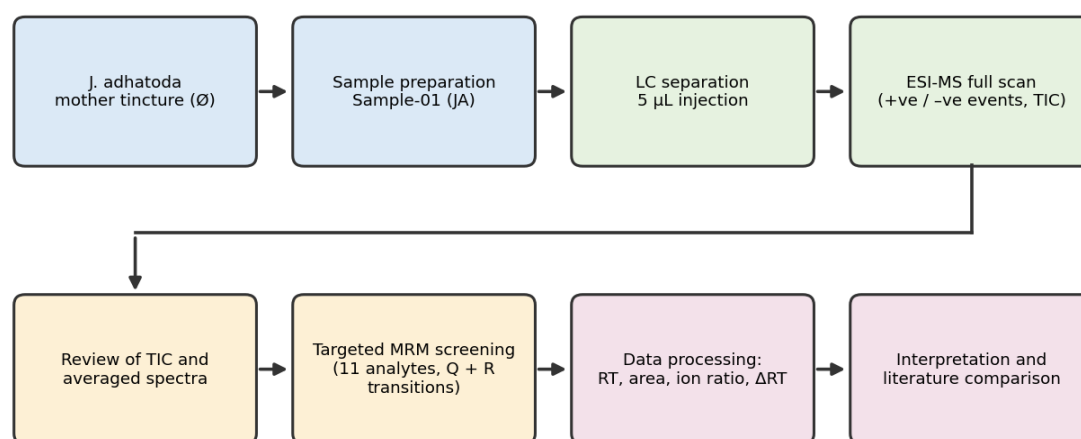


Figure 1 – Flowchart of the LC–MS methodology.

Inference: The workflow runs from a general full-scan survey to targeted MRM, so each identification rests on two kinds of evidence.

$\text{Ion ratio} = \text{qualifier area} \div \text{quantifier area} \times 100$; $\Delta\text{RT} = |\text{RT}_{\text{quantifier}} - \text{RT}_{\text{qualifier}}|$; $\text{relative signal share} = \text{peak area} \div \text{summed peak area of the nine targeted alkaloids} \times 100$. Two transitions with consistent RT and ion ratio provide supporting identification evidence [16]; because calibration and reference-standard results were unavailable, identifications remain putative and were graded High, Good, Moderate, Tentative or Not confirmed (grading limits set for this report).

RESULTS AND OBSERVATION

The TIC (Figure 2) peaked at 7.94×10^8 counts in a broad late hump (about 33–35 min); a group of peaks at 11–15 min (up to about 2.5×10^8) lies in the alkaloid region.

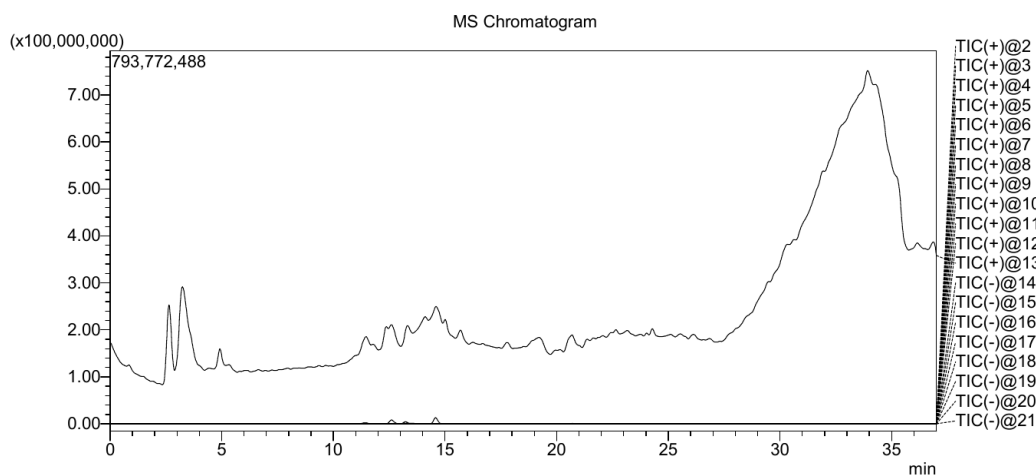


Figure 2 – Total ion chromatogram of Sample-01 (JA), TIC(+@2–@13 and TIC (–)@14–@21.

Inference: The 11–15 min group coincides with the RT of vasicinol, vasicine, vasicoline and vasicinone (Table 1); the late hump is probably lipophilic matrix and was not assigned.

Averaged full-scan spectra gave base peaks at m/z 205 (11.1–11.7 min), 189 (12.2–13.0 min), 219 (13.1–13.6 min), 203 (14.3–14.9 min) and 352 (20.3–21.0 min), matching the precursor ions of vasicinol, vasicine, vasicoline, vasicinone and 7-hydroxyadhatodine. MRM results are in Table 1 and Figure 3.

Table 1 – MRM results for eleven analytes in Sample-01 (JA) (m/z transitions; orientin and vitexin in negative mode, others in positive mode).

Compound	RT (min)	Quantifier	Qualifier	Peak area	Ion ratio (%)	Δ RT (min)	Confidence
Vasicine	12.60	189.1>171.1	189.1>143.1	103,383,823	19.3	0.001	High
Vasicinone	14.58	203.1>185.1	203.1>82.1	156,948,424	12.6	0.000	High
Vasicinol	11.43	205.1>187.1	205.1>159.1	32,479,228	22.8	0.002	High
Vasicoline	13.23	219.1>201.1	219.1>174.1	53,270,124	2.3	0.001	High
Adhatodine	20.52	338.2>292.2	338.2>246.2	920,231	2.3	0.001	Moderate
Anisotine	14.65	276.1>258.1	276.1>230.1	30,558	82.2	0.099	Tentative (trace)
Deoxyvasicine	19.86	173.1>155.1	173.1>128.1	205,708	15.9	0.002	Tentative
7-Hydroxyvasicolinone	19.63	322.1>262.1	322.1>290.1	653,788	66.3	0.001	Good
7-Hydroxyadhatodine	20.71	352.2>274.2	352.2>306.2	12,767,821	9.1	0.003	High
Orientin	21.52	447.1>327.1	447.1>357.1	26,884	6.0	0.278	Not confirmed
Vitexin	15.76	431.1>311.0	431.1>341.0	71,302	21.6	0.008	Tentative

Six analytes reached High or Good confidence, with $\Delta RT \leq 0.003$ min and clear qualifier peaks; adhatodine was Moderate; deoxyvasicine, anisotine and vitexin were tentative, and orientin was not confirmed (ΔRT 0.278 min). Alkaloid peak areas ranged from approximately 2.7×10^4 to 1.57×10^8 counts, a span of about 3.8 orders of magnitude.

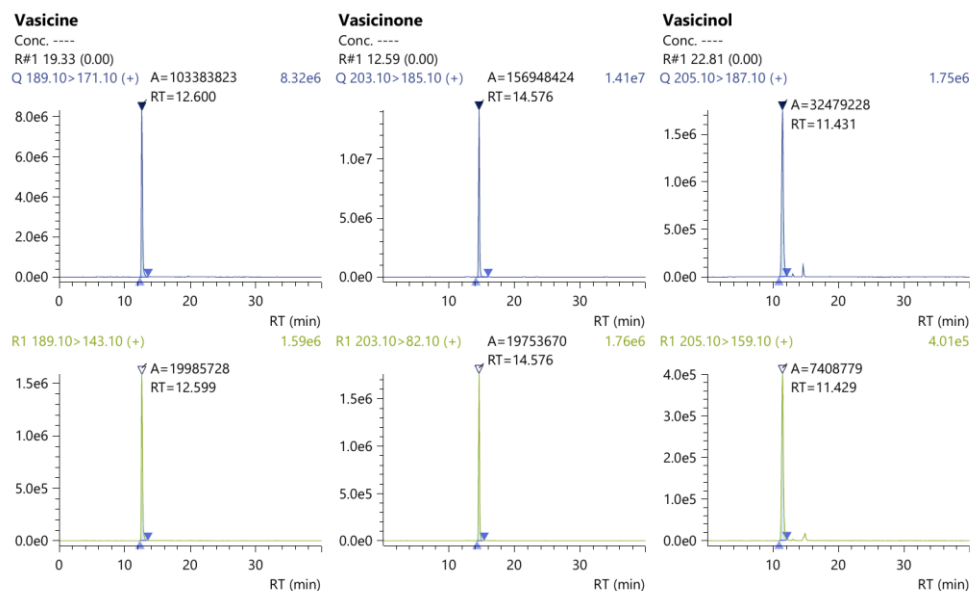


Figure 3 – MRM chromatograms of vasicine, vasicinone and vasicinol: quantifier (upper) and qualifier (lower) traces.

Each of the three highlighted alkaloids gives a sharp peak in both transitions at the same RT (12.60, 14.58 and 11.43 min), supporting their putative identification.

Table 2 – Relative abundance of the nine alkaloids (share of summed quantifier peak area, 360,659,705).

Rank	Alkaloid	RT (min)	Share (%)	Cumulative (%)
1	Vasicinone	14.58	43.52	43.52
2	Vasicine	12.60	28.67	72.18
3	Vasicoline	13.23	14.77	86.95
4	Vasicinol	11.43	9.01	95.96
5	7-Hydroxyadhatodine	20.71	3.54	99.50
6	Adhatodine	20.52	0.26	99.75
7	7-Hydroxyvasicolinone	19.63	0.18	99.93
8	Deoxyvasicine	19.86	0.057	99.99
9	Anisotine	14.65	0.008	100.00

Vasicinone and vasicine account for 72.2% of the alkaloid signal and, with vasicoline and vasicinol, 96.0%. Vasicinone's signal was 1.52 times vasicine's signal. Late-eluting alkaloids account for 4.0% of the signal (7-hydroxyadhatodine 3.5%). These percentages compare peak-area signals, not compound amounts, because analyte-specific response factors were not established.

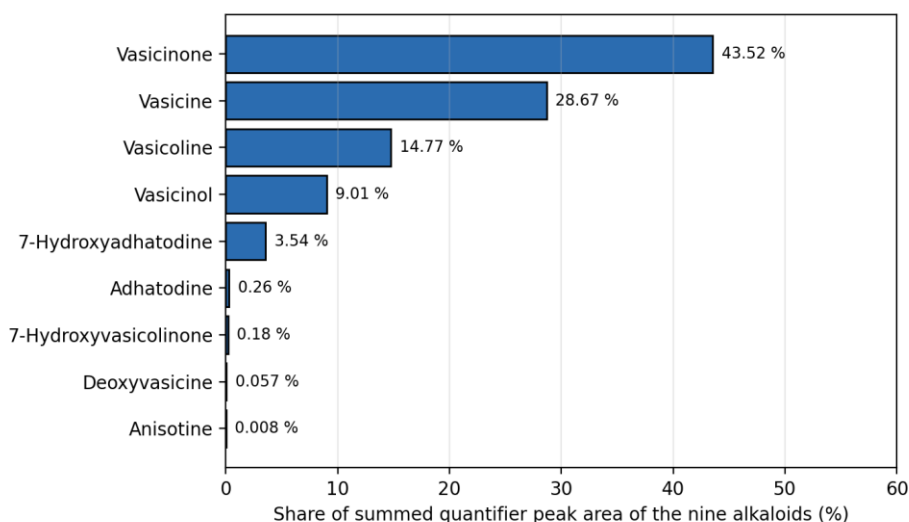


Figure 4 – Share of each alkaloid in the summed quantifier peak area.

Four compounds account for nearly all of the measured alkaloid signal; the other five contribute 3.5% or less each. These peak-area proportions should not be interpreted as concentration or amount without analyte-specific calibration.

DISCUSSION

Full-scan and MRM data agree: base peaks at m/z 205, 189, 219 and 203 fall in the same windows as the MRM peaks of the four main alkaloids, strengthening their putative identification. The four dominant alkaloids account for 96.0% of the measured alkaloid signal. This is a comparison of detector responses, not a direct comparison of compound amounts, because response factors may differ between analytes.

Vasicinone gave a stronger signal than vasicine (signal ratio 1.52), whereas vasicine is usually described as the main leaf alkaloid and was quantified at about 1.5–1.8 g per 100 mL in marketed preparations [9]. Possible explanations include oxidation of vasicine to vasicinone during extraction or storage [4], different MRM response factors, and differences in plant source or extraction; the present data cannot distinguish among these possibilities.

Earlier HPLC, CE, HPTLC and UPLC/Q-TOF-MS methods quantify one or two alkaloids in leaves, extracts or formulations [4–7,9,10], whereas this study screens eleven analytes with two kinds of MS evidence in the tincture itself; it is broader but less quantitative. Because vasicinone, vasicoline and vasicinol contribute 67.3 % of the alkaloid signal, total alkaloids “as vasicine” [3] cover several distinct compounds, and a fingerprint of the four dominant alkaloids could support batch identity checks.

Limitations: one sample and one injection; no calibration or reference standards, so identifications are putative; analyte-specific response factors and matrix effects were not evaluated; the deoxyvasicine transition (m/z 173>155, a loss of 18 Da from a compound with no oxygen) needs verification; the late matrix hump was not characterised. The available record also omits key chromatographic and mass-spectrometric settings and sample/batch details, limiting reproducibility. H0 is rejected for the main alkaloids; H1 is partly supported, since vasicinone gave the strongest signal, not vasicine.

CONCLUSION

The mother tincture of *Justicia adhatoda* showed a signal profile dominated by vasicinone, vasicine, vasicoline and vasicinol (96.0% of summed alkaloid peak area), with 7-hydroxyadhatodine and trace signals from other targets. Full-scan and MRM evidence agree for the main alkaloids, but identifications remain putative without reference standards. Peak-area shares are not compound amounts. Calibrated analysis with reference standards, replicate injections, complete method reporting and several batches is recommended before these alkaloids are adopted as quality markers.

SUMMARY

One homoeopathic mother tincture of *J. adhatoda* was analysed by full-scan LC–MS and MRM for eleven analytes. The alkaloid region lay at 11–15 min, and base peaks at m/z 205, 189, 219 and 203 matched the RT windows of vasicinol, vasicine, vasicoline and vasicinone. MRM signals were observed for all nine targeted alkaloids, but deoxyvasicine and anisotine were tentative; therefore, all nine should not be described as confirmed. Four alkaloids accounted for 96.0% of the measured alkaloid signal, with vasicinone giving the strongest signal (43.5% of summed peak area). Orientin was not confirmed and vitexin was tentative. Signal shares do not establish relative amounts.

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