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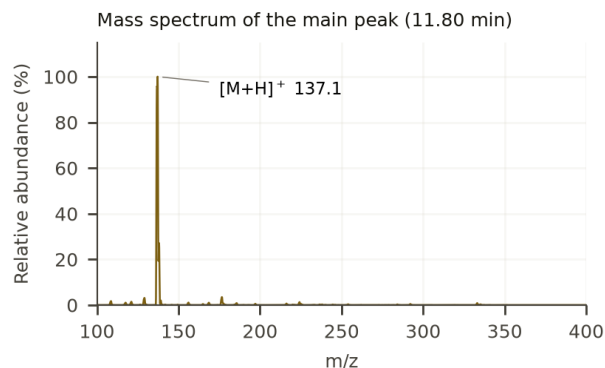
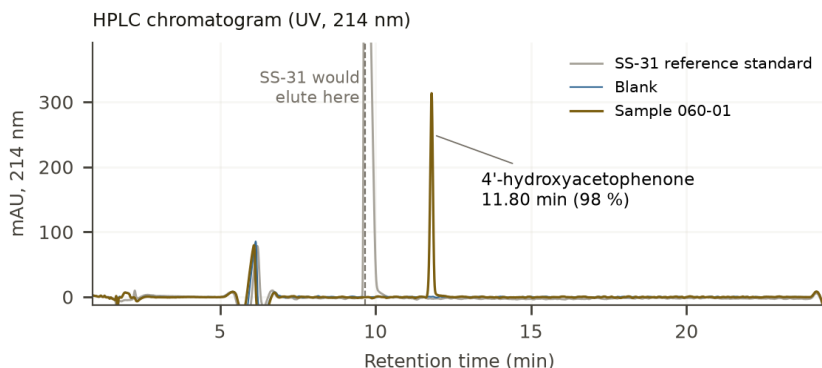
SS-31 (Elamipretide) | 100 mg vial | lot Lot 001 | full identity investigation

Customer	Jeremy D Hughes
Order	GSA-2026-000060
Sample	060-01
Label	"SS-31 (Elamipretide) 100 mg" (Signal Matrix), lot "Lot 001"
Prepared	18 Oct 2026
Tested	19-21 Oct 2026 (9 sample injections, each after a blank)
Tests	Identity, purity, amount of the main component



The sample as received

SS-31 (ELAMIPRETIDE) ABSENT Not detected < 0.001 % of the label (< 1 µg per vial), targeted SIM	MAIN COMPONENT CONSISTENT 4'-Hydroxy-acetophenone consistent with; C₈H₈O₂, 98 % of UV area	AMOUNT (ESTIMATE) ESTIMATE About 15 mg per vial (about 15 % of the labelled 100 mg); UV estimate	EVIDENCE INFO 6 independent lines UV spectrum, mass, isotopes, ESI+ and ESI- fragments, acidity
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Summary

The vial does not contain detectable SS-31 (elamipretide). In a targeted selected-ion measurement the SS-31 signal of the sample equals the instrument blank; any SS-31 present is below 0.001 % of the labelled 100 mg (less than about 1 µg per vial).

The main content of the vial is consistent with 4'-hydroxyacetophenone (C₈H₈O₂, molecular mass 136; a small aromatic molecule, not a peptide). It is 98 % of the UV-absorbing material, estimated at roughly 15 mg per vial. Six independent lines of evidence agree (UV spectrum, molecular ion, isotope pattern, positive- and negative-mode fragment ions, and the acidity of the molecule). No reference sample of 4'-hydroxyacetophenone was injected, so close positional isomers are not fully excluded.

The rest of the labelled 100 mg is not accounted for by UV-absorbing material and was not identified. Net peptide content does not apply because no peptide was found.

1. Tests performed and results

#	Test	Run, date, volume	Result	Conclusion
1	Initial LC-UV-MS screen	WL1-10, 19 Oct, 1 µL	Main UV component at 11.80 min. This injection directly followed an SS-31 reference injection.	Repeated after a blank (test 2)
2	LC-UV profile (DAD, 214 nm) and ESI+ full scan m/z 100-1000	WL3-16 after blank WL3-15, 20 Oct, 1 µL	One main component at 11.80 min, 98.4 % of UV area; 5 minor components each < 0.5 %. SS-31 ions (m/z 640.4 / 320.7 / 214.1) at blank level; no UV peak at 9.66 min.	SS-31 not detected; one dominant small molecule

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#	Test	Run, date, volume	Result	Conclusion
3	Targeted SS-31 selected-ion monitoring	WL3-22 vs blank WL3-21, 20 Oct, 2 µL	m/z 640.4: 0.011 ng (sample) vs 0.012 ng (blank); detection limit about 0.002 ng (table 2).	SS-31 < 0.001 % of the label
4	UV-DAD spectrum of the main component	WL3-16	Absorbance maxima 276 and 218 nm, minimum 236 nm, absorbance to about 310 nm. SS-31 itself absorbs mainly below 230 nm.	Consistent with a para-hydroxy aryl ketone chromophore
5	Molecular ion	WL3-16 (ESI+), WL3-32 (ESI-)	[M+H] ⁺ m/z 137.1 and [M-H] ⁻ m/z 135; no sodium or potassium adducts.	Molecular mass 136
6	Isotope pattern (SIM, low volume)	WL3-37, 21 Oct, 0.1 µL	M+1 8.83 %, M+2 0.73 % (theory for C ₈ H ₉ O ₂ ⁺ : 8.83 %, 0.75 %).	Formula C ₈ H ₈ O ₂
7	ESI+ product ions of m/z 137	WL3-30 vs blank WL3-29, 21 Oct, 2 µL	m/z 43 (base, acetyl cation); precursor 36 %; m/z 95 0.2 %.	Acetyl group
8	ESI- full scan m/z 50-600	WL3-32 vs blank WL3-31, 21 Oct, 2 µL	[M-H] ⁻ m/z 135 is the base ion, more than 300 times the blank.	Acidic (phenol-type) proton
9	ESI- product ions of m/z 135	WL3-38, 21 Oct, 2 µL	m/z 120 (loss of CH ₃), 93 (loss of ketene), 92 (loss of acetyl); no loss of CO ₂ (m/z 91) or CO (m/z 107).	Aryl methyl ketone on a phenol; not an acid or aldehyde
10	Candidate comparison	tests 4-9	11 candidate compounds or compound classes, including SS-31, compared against all data (table 5).	4'-Hydroxyacetophenone fits all data; only the 3'-isomer is not excluded outright
11	Amount estimate (UV, 280 nm)	WL1-10	About 0.15 mg/mL in the working solution, i.e. about 15 mg per vial if dissolved as planned.	Estimate (literature absorptivity)
12	Energy-resolved MRM fingerprints	WL3-33 to WL3-36, 21 Oct	Acquired as a supplementary fingerprint.	Not evaluated in this report
13	Final blank	WL3-39, 21 Oct	m/z 137 at most 0.01 % of the sample signal; SS-31 ions at background level.	Clean

Run codes refer to the injections listed in section 7. Volumes are injection volumes of the 1.0 mg/mL (planned) working solution.

2. SS-31 (elamipretide): not detected

SS-31 (D-Arg-Dmt-Lys-Phe-NH₂, C₃₂H₄₉N₉O₅, monoisotopic mass 639.39 Da) is detected in this method as [M+H]⁺ m/z 640.4, [M+2H]²⁺ m/z 320.7 and [M+3H]³⁺ m/z 214.1 and elutes at 9.66 min (UV). Retention time, ions and response were established by injecting an SS-31 reference standard in the same sequences.

Initial screen (19 Oct). The first injection of the sample directly followed an SS-31 reference injection, so the sample was re-analysed after a blank.

Repeat after a blank (20 Oct, 1 µL). No UV peak above 0.01 % of the UV area at the SS-31 retention time (figure 1). In the full-scan MS data the m/z 640.4 signal in the SS-31 window equals the preceding blank (8.1 x 10⁴ vs 7.7 x 10⁴ counts; the SS-31 reference at 0.5 µL gives 2.5 x 10⁹), and the same holds for m/z 320.7 and 214.1 (figure 2a).

Targeted selected-ion monitoring (20 Oct, 2 µL). If the vial contained SS-31 as labelled, this injection would hold about 2000 ng. The measured signal equals the blank on all three SS-31 ions (table 2, figure 2b). The small signal common to blank and sample is SS-31 background from reference injections earlier in the sequence. The upper bound, 0.011 ng per 2000 ng, is **less than 0.001 % of the label, i.e. less than about 1 µg of SS-31 per vial**.

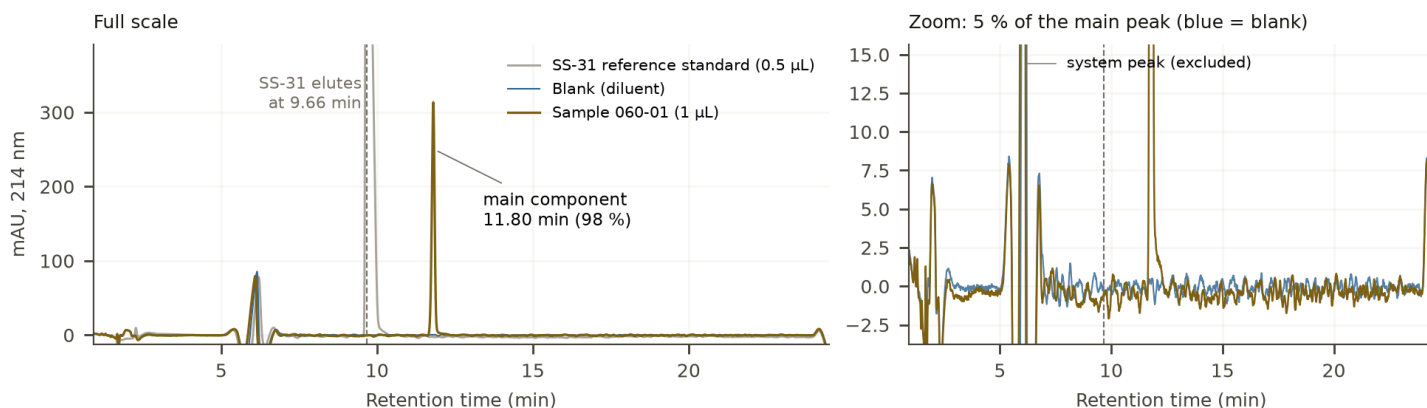


Figure 1. UV chromatogram at 214 nm of sample 060-01 (1 µL, 20 Oct), its blank and an SS-31 reference standard injection (0.5 µL). Baseline drift of the gradient removed using the blank. The sample has no peak at the SS-31 retention time.

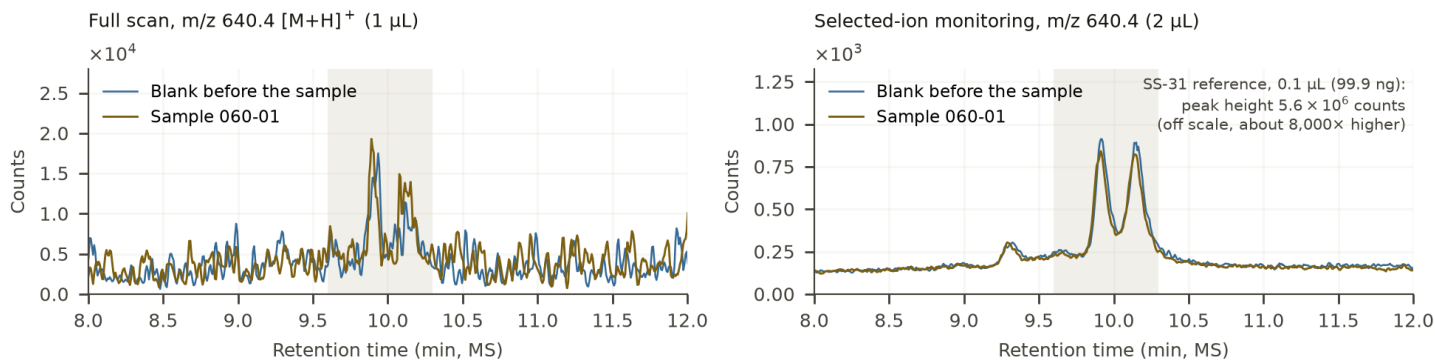


Figure 2. SS-31 [M+H]⁺ trace (m/z 640.4). (a) Full scan, sample after a blank. (b) Selected-ion monitoring at 2 µL. Shaded: SS-31 retention window (MS retention times run about 0.2-0.3 min later than UV). The sample trace (gold) matches the blank (blue) in both.

Table 2. SS-31 by selected-ion monitoring (ng SS-31 on column, from the reference response at 0.1 µL = 99.9 ng)

Ion	Sample 060-01 (2 µL)	Blank before the sample	Detection limit (3 × noise)	Result
m/z 640.4 [M+H] ⁺	0.0108	0.0118	0.0023	equal to blank
m/z 320.7 [M+2H] ²⁺	0.0142	0.0147	0.0044	equal to blank
m/z 214.1 [M+3H] ³⁺	0.0014	0.0024	0.0018	below detection limit

3. Identification of the main component (11.80 min)

The component at 11.80 min accounts for 98.4 % of the UV area at 214 nm. It elutes at about 22 % acetonitrile, which indicates a moderately polar small molecule. Each test below was chosen to tell apart the compounds that could give the same molecular mass.

3.1 UV spectrum

The diode-array spectrum (figure 3a) has a strong band at 276 nm, a second band at 218 nm, a minimum at 236 nm and absorbance extending to about 310 nm. This is a conjugated chromophore: a ketone conjugated with a para-hydroxy benzene ring (literature maximum of 4'-hydroxyacetophenone in neutral or acidic water: 275-278 nm [2]). The 2'-isomer has an additional band near 329 nm, which is absent. A peptide such as SS-31 absorbs mainly below 230 nm (peptide bonds), with only a weak aromatic band near 280 nm (dashed curve).

3.2 Molecular ion and formula

The positive ion [M+H]⁺ m/z 137.1 (figure 3b) and the negative ion [M-H]⁻ m/z 135 (figure 4a) both give a molecular mass of 136. No sodium (m/z 159) or potassium (m/z 175) adducts were seen. The isotope pattern was measured by selected-ion monitoring at 0.1 µL so the signal stayed in the detector's linear range; the M+1 ratio was constant across the top half of the peak (8.4-9.1 %). The measured ratios (M+1 8.83 %, M+2 0.73 %) match C₈H₉O₂⁺ exactly and exclude the nitrogen-containing and C₉ formulas of the same nominal mass (table 3, figure 4b).

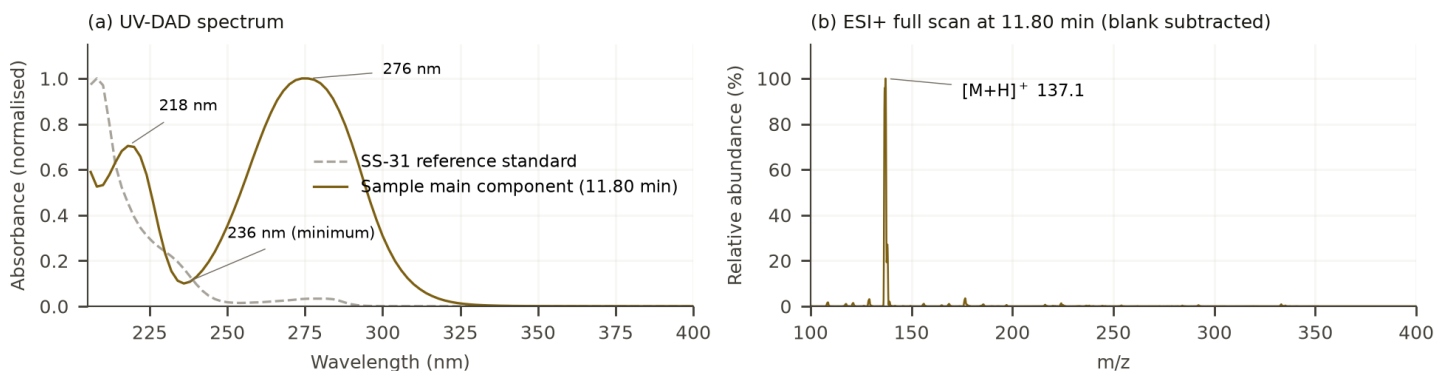


Figure 3. (a) UV spectra (normalised) of the sample main component and of SS-31. (b) ESI+ mass spectrum at the main peak; the apex of m/z 137 is too strong at 1 µL for isotope ratios, so they were measured separately at 0.1 µL (figure 4b).

Table 3. Isotope ratios of the m/z 137 ion: measured vs theory for formulas of nominal mass 137

Ion formula	Examples	M+1 (% of M)	M+2 (% of M)	Verdict
Measured	sample 060-01, SIM at 0.1 µL (WL3-37)	8.83	0.73	
C ₈ H ₉ O ₂ ⁺	4'-hydroxyacetophenone and other C ₈ H ₈ O ₂ isomers	8.83	0.75	fits
C ₇ H ₉ N ₂ O ⁺	e.g. aminobenzamides	8.44	0.52	does not fit
C ₈ H ₁₃ N ₂ ⁺	e.g. betahistine	9.53	0.41	does not fit
C ₉ H ₁₃ O ⁺	e.g. propylphenols	9.92	0.65	does not fit
C ₅ H ₅ N ₄ O ⁺	hypoxanthine	6.96	0.42	does not fit

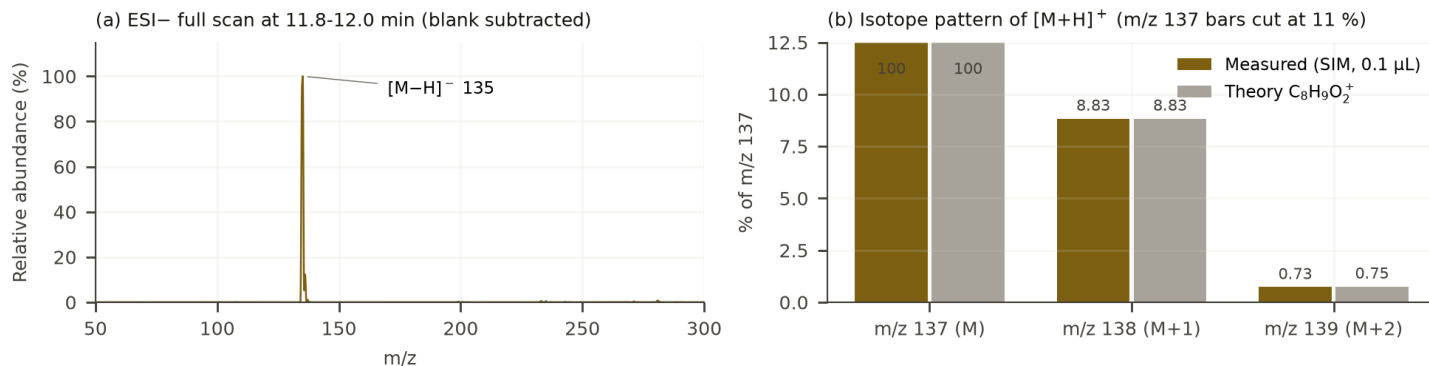


Figure 4. (a) ESI- mass spectrum at the main peak: the deprotonated molecule m/z 135 is the base ion, so the molecule carries an acidic proton. (b) Isotope pattern of $[M+H]^+$ measured at 0.1 μ L, compared with theory for $C_8H_9O_2^+$.

3.3 Fragment ions

Product-ion spectra were recorded in both polarities (figure 5, table 4). In positive mode the protonated molecule breaks almost entirely into the acetyl cation CH_3CO^+ (m/z 43). In negative mode the deprotonated molecule loses a methyl radical (m/z 120), ketene ($CH_2=C=O$, m/z 93, a phenoxide ion) and an acetyl radical (m/z 92). There is no loss of CO_2 (m/z 91), which a carboxylic acid would show, and no loss of CO (m/z 107), which a phenolic aldehyde would show. Together these show an acetyl group bonded to a phenol ring: a hydroxyacetophenone. Published spectra of 4'-hydroxyacetophenone list m/z 93 as the main negative-mode product [3] and m/z 95 as the main positive-mode product at low collision energy [4]; at the energy used here the acetyl ion dominates and m/z 95 is weak (0.2 %).

Table 4. Product ions (relative abundance: % of base peak in ESI+, % of precursor in ESI-)

Mode	Precursor	Product m/z	Abundance %	Interpretation
ESI+	137 $[M+H]^+$	43	100	CH_3CO^+ (acetyl cation)
		137	36	surviving precursor
		95	0.2	loss of ketene (protonated phenol)
		91 / 107	0.03 / 0.00	absent: no CO_2 or CO loss
ESI-	135 $[M-H]^-$	120	6.0	loss of CH_3 radical
		93	5.8	loss of ketene (phenoxide)
		92	5.0	loss of acetyl radical
		134	6.2	not assigned
		91 / 107	0.03 / 0.00	absent: no CO_2 or CO loss

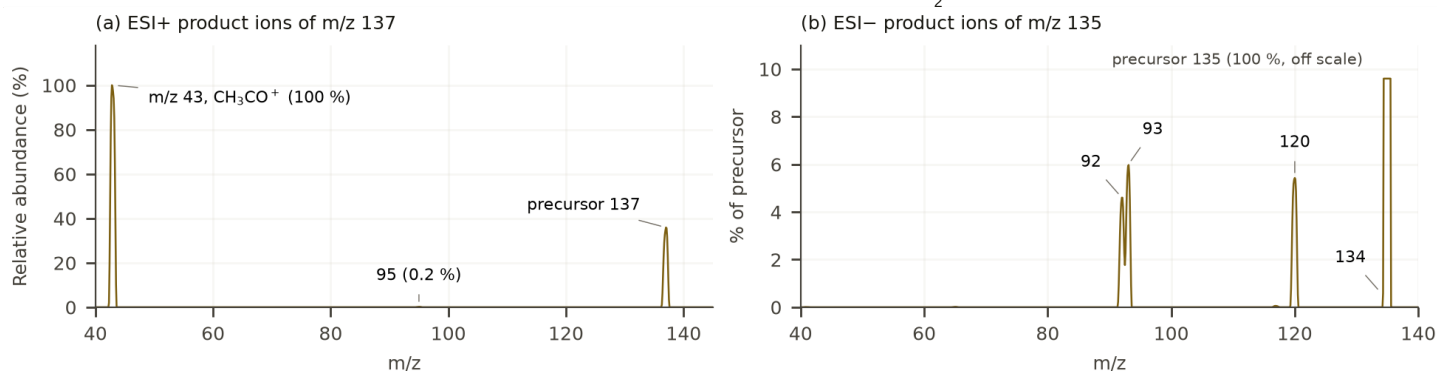


Figure 5. (a) ESI+ product ions of m/z 137 (WL3-30). (b) ESI- product ions of m/z 135 (WL3-38), shown up to 10 % of the precursor.

3.4 Candidates compared



Table 5. Candidate compounds and the observation that decides each

Candidate	Formula	What it would show	Observed	Verdict
4'-Hydroxyacetophenone	C ₈ H ₈ O ₂	phenol ([M-H] ⁻), acetyl (m/z 43; ketene loss to 93), para band near 275 nm	all observed	Consistent
2'-Hydroxyacetophenone	C ₈ H ₈ O ₂	additional UV band near 329 nm	absent	Not consistent
3'-Hydroxyacetophenone	C ₈ H ₈ O ₂	same ions; no para-type conjugation in the UV	UV favours the 4'-isomer	Not excluded without a reference
4-Methoxybenzaldehyde (anisaldehyde)	C ₈ H ₈ O ₂	no acidic proton (no [M-H] ⁻); CO loss	strong [M-H] ⁻ ; no CO loss	Not consistent
Phenyl acetate, methyl benzoate	C ₈ H ₈ O ₂	no acidic proton; weak benzenoid UV	strong [M-H] ⁻ ; strong 276 nm band	Not consistent
Phenylacetic acid, toluic acids	C ₈ H ₈ O ₂	CO ₂ loss from [M-H] ⁻ (m/z 91)	m/z 91 at 0.03 %	Not consistent
Hydroxy-methylbenzaldehydes	C ₈ H ₈ O ₂	CO loss from [M-H] ⁻ (m/z 107); no acetyl ion	m/z 107 absent; m/z 43 base	Not consistent
Hypoxanthine	C ₅ H ₄ N ₄ O	M+1 about 7.0 %; elutes near the void	M+1 8.83 %; 11.80 min	Not consistent
Betahistine	C ₈ H ₁₂ N ₂	M+2 0.41 %; no phenol; elutes early	M+2 0.73 %	Not consistent
Propylphenols and other C ₉ H ₁₂ O	C ₉ H ₁₂ O	M+1 about 9.9 %; no acetyl group	M+1 8.83 %; m/z 43 base	Not consistent
SS-31 (elamipretide)	C ₃₂ H ₄₉ N ₉ O ₅	m/z 640.4 / 320.7 / 214.1 at 9.66 min	not detected	Absent

3.5 Amount

Using the UV peak area at 280 nm and a literature molar absorptivity of about $1.4 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$ for 4'-hydroxyacetophenone, the working solution contains about 0.15 mg/mL of the compound. If the whole vial was dissolved to the planned 1.0 mg/mL, this is about **15 mg per vial**, roughly 15 % of the labelled mass. This is an estimate: it was not calibrated against the compound itself, and it assumes the vial was dissolved as planned.

3.6 Conclusion

The main component of the vial is consistent with 4'-hydroxyacetophenone (1-(4-hydroxyphenyl)ethanone, piceol; CAS 99-93-4; C₈H₈O₂). All six lines of evidence agree. Of the alternatives in table 5, only 3'-hydroxyacetophenone is not excluded outright, and the strong single band at 276 nm favours the 4'-isomer. The assignment was made without injecting a reference sample of the compound; GSA can confirm it against an authentic reference on request, which would also settle the 3'-isomer question.

4. Other observations

Table 6. UV components of the sample at 214 nm (WL3-16, blank-subtracted, % of counted UV area)

Retention time (min)	Area %	Comment
11.80	98.38	main component (section 3)
7.37	0.45	not identified
7.09	0.40	not identified
7.70	0.36	not identified
10.07	0.25	not identified
21.82	0.16	not identified
9.76	0.01	baseline noise near the SS-31 retention time

Area % at 214 nm is not a mass fraction: different compounds absorb differently. None of the minor components shows SS-31 ions.

- A UV-only peak at 5.90 min (absorbs only below about 230 nm, no response in positive mode, and not trifluoroacetate: no m/z 113 in negative mode) is also seen in other injections of the sequence. It is treated as a diluent/system peak and excluded from table 6, as is the solvent front at 1.66 min.
- Sugar-alcohol-type ions at the solvent front.** In negative mode, ions at m/z 181 and 227 at 1.5 min were 160 and 620 times the blank (figure 6). This is the pattern of a hexitol such as mannitol or sorbitol ([M-H]⁻ and formate adduct). Hexitols are common bulking agents in freeze-dried products, but matching positive-mode ions were also present in other solutions prepared in the laboratory the same day, so this signal cannot be attributed to the vial with certainty. Hexitols do not absorb UV and were not quantified.
- The UV-absorbing content (about 15 mg) accounts for only part of the labelled 100 mg. The remainder was not identified: it may be material without UV absorbance (such as a sugar alcohol or salt) or a lower fill than labelled; the vial fill weight was not recorded.
- Energy-resolved MRM fingerprints (WL3-33 to WL3-36) were acquired as supplementary data. They were not needed for the conclusions and are not evaluated here.

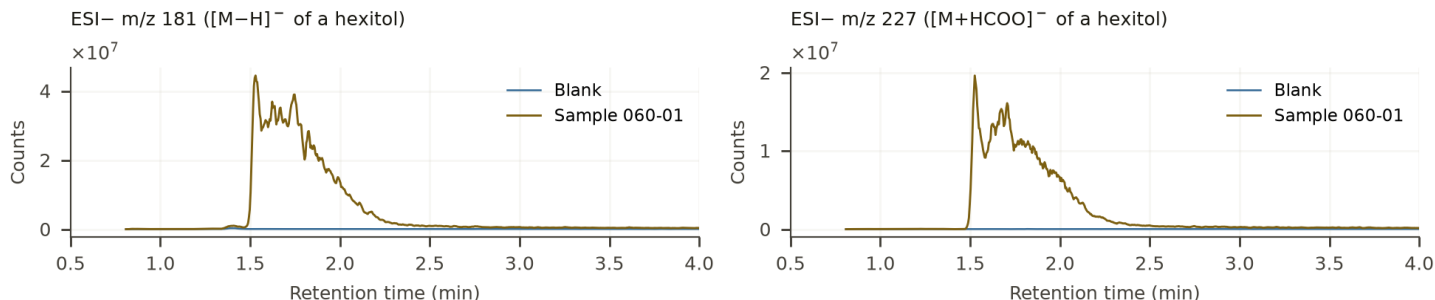


Figure 6. ESI- traces of hexitol-type ions at the solvent front (WL3-32 vs blank WL3-31).

5. Limitations

- Identification by retention time, UV spectrum and mass spectrometry (including fragment ions); no reference sample of the proposed compound was injected.
- The amount of the main component is an estimate from a literature absorptivity (section 3.5) and assumes the vial was dissolved as planned.

6. Methods

Agilent 1290 Infinity II LC (G7120A pump, G7167B multisampler, G7116B column compartment, G7115A diode-array detector) with a G6490A triple-quadrupole mass spectrometer (electrospray). Column Polaris C18-A, 2.0 x 150 mm, 5 μ m, 45 $^{\circ}$ C. Mobile phase A water + 0.1 % formic acid, B acetonitrile + 0.1 % formic acid, 0.3 mL/min, 0-50 % B from 3 to 23 min. Every MS method used the same chromatography, and every sample injection followed a blank (diluent) injection with the same method.

Method	Acquisition	Purpose
GSA_UNIV_VOID_45C_v2	DAD 190-400 nm; ESI+ full scan m/z 100-1000	UV profile, SS-31 screen, molecular ion
GSA_SS31_SIM_VOID_v1	ESI+ SIM m/z 640.4, 320.7, 214.1 (and 137.1)	trace SS-31 and detection limit
GSA_UNK_PI_VOID_v2	ESI+ product-ion scan of m/z 137.1, m/z 40-145	positive fragments
GSA_UNK_NEG_VOID_v2	ESI- full scan m/z 50-600	[M-H] ⁻ , acidity, hexitol ions
GSA_UNK_ISO_SIM_v1	ESI+ SIM m/z 121.1-176.6 (9 ions, 40 ms), 0.1 μ L	isotope ratios and adduct check
GSA_UNK_PI_NEG_v1	ESI- product-ion scan of m/z 135, m/z 40-140	negative fragments
GSA_UNK_MRM_POS_v1 / NEG_v1	MRM breakdown series	supplementary (not evaluated)

Processing: native Agilent data read with the vendor data-access library; UV traces corrected for gradient drift using the matched blank; peaks integrated over fixed windows set before processing; MS spectra averaged over the peak and background-subtracted with a window just before the peak; SIM amounts in ng from the SS-31 reference response at 0.1 μ L; detection limit = 3 x baseline noise.

7. Injections of this sample and its blanks

Started	Run	Method (GSA...)	Inj. μ L	Purpose
19 Oct 17:54	WL1-10	UNIV_VOID_v2	1	initial screen
20 Oct 16:45	WL3-15	UNIV_VOID_v2	2	blank (diluent)
20 Oct 17:19	WL3-16	UNIV_VOID_v2	1	UV profile, full scan
20 Oct 17:54	WL3-21	SS31_SIM_v1	2	blank (diluent)
20 Oct 18:29	WL3-22	SS31_SIM_v1	2	SS-31 SIM
21 Oct 12:26	WL3-29	UNK_PI_v2	2	blank (diluent)
21 Oct 13:00	WL3-30	UNK_PI_v2	2	ESI+ product ions
21 Oct 13:35	WL3-31	UNK_NEG_v2	2	blank (diluent)
21 Oct 14:10	WL3-32	UNK_NEG_v2	2	ESI- full scan
21 Oct 14:45	WL3-33	UNK_MRM_POS_v1	2	blank (diluent)
21 Oct 15:19	WL3-34	UNK_MRM_POS_v1	2	MRM (supplementary)
21 Oct 15:54	WL3-35	UNK_MRM_NEG_v1	2	blank (diluent)
21 Oct 16:29	WL3-36	UNK_MRM_NEG_v1	2	MRM (supplementary)
21 Oct 17:04	WL3-37	UNK_ISO_SIM_v1	0.1	isotope pattern
21 Oct 17:38	WL3-38	UNK_PI_NEG_v1	2	ESI- product ions
21 Oct 18:13	WL3-39	UNIV_VOID_v2	2	final blank

SS-31 reference standard injections (same sequences) supplied the retention time, the ion pattern and the SIM response for the detection limit. Raw data files are retained by Gold Standard Analytics. Start times are the creation times of the data files.

8. About 4'-hydroxyacetophenone

4'-Hydroxyacetophenone (piceol) is a commercially available aromatic chemical. Among other uses it is a cosmetic ingredient (INCI name hydroxyacetophenone, used as an antioxidant and preservative booster) [5] and is listed as Paracetamol Impurity E in the European Pharmacopoeia [6]. It is not a peptide and has no structural relationship to SS-31. This section is background information only.

References

- [1] PubChem CID 7469, 4'-Hydroxyacetophenone. <https://pubchem.ncbi.nlm.nih.gov/compound/7469>
- [2] Givens R.S. et al. (2011), PubMed Central PMC3891043 (cited for the UV maxima of 4'- and 2'-hydroxyacetophenone in water). <https://pmc.ncbi.nlm.nih.gov/articles/PMC3891043>
- [3] MassBank records LU021451-LU021456, 4'-hydroxyacetophenone, ESI- MS/MS (precursor m/z 135.045).
- [4] ContaminantDB MS/MS spectra 1476101 and 1476102, 4'-hydroxyacetophenone, ESI+ (precursor m/z 137.06). https://contaminantdb.ca/spectra/ms_ms/1476101
- [5] US Patent 10,981,854 B2 (cited for the cosmetic use of hydroxyacetophenone). <https://patents.google.com/patent/US10981854B2/en>
- [6] Paracetamol EP Impurity E, 1-(4-hydroxyphenyl)ethanone (reference material listing). <https://www.clearsynth.com/en/CST26516.html>

APPROVED FOR RELEASE

Signed electronically by a Gold Standard Analytics lab technician at release. The signature, date and verification code are on the release record, the last page of this certificate.

Customer and order	Jeremy D Hughes · GSA-2026-000060
Sample and report	060-01 · Certificate of Analysis, vial labelled SS-31
Report reference	GSA-COA-000060-06001-20261026
Certificate accession	GSA-2026-853724
Released and signed by	Ian Ebert, Laboratory Director
Date and time (UTC)	2026-10-26 17:43:40Z
Signature meaning	Approved for release. The signer reviewed this report and approved its release to its customer.
Approval reference	5c1f1ffd-55c3-4ba0-a3ad-1f5c967e1322
Electronic signature	Signed electronically in a signed-in GSA staff account.
Original report SHA-256	4e7762bf546bd4a703d154d50be55b65a0c755dbea6c700cc5196b1766770260
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Signature

A handwritten signature in black ink, appearing to read 'Ian Ebert', written in a cursive style.

Ian Ebert, Laboratory Director